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Abstract

ADAPTIVE FORAGING IN A GENERALIST PREDATOR: IMPLICATIONS OF HABITAT STRUCTURE, DENSITY, PREY AVAILABILITY AND NUTRIENTS

by Jason M. Schmidt

Adaptive foraging is a recent theoretical synthesis linking foraging decisions to the structure and functioning of ecological communities. However, empirical research is required to characterize the consequences of different environmental challenges on organisms foraging behavior. The goal of this dissertation was to explore ecological factors that are responsible for variation in the foraging behavior of a common generalist predator, the wolf spider Pardosa milvina. I conducted four studies to investigate aspects of the adaptive foraging framework, space use and prey selection. I first explored the how habitat structure affects density and foraging ability. Spiders preferred areas containing more structural features, but contrary to predictions, they preferred patches low in prey. Foraging rate was reduced by some habitat features, and habitat structure lowered interference levels between spiders. I examined the role of prey abundance and predator abundance on interference determined by changes in patch leaving frequency and in their functional response. While prey abundance had strong effects on the tendency to leave patches, indicating the importance of prey to predator patch use, increasing the abundance of predators led to increased dispersal rates and interference. I used a two-pronged approach to understanding prey selection in this spider. In one set of experiments I measured changes in consumption of a target prey group in the field using a molecular probe. Although there was variation in the proportion of spiders testing positive for prey, the consumption of prey did not follow strict frequency dependence expected for generalist species. In a second set of experiments I tested for one prey characteristic, nutrient quality, to help explain predation rate and prey choice. The functional response of spiders to different prey nutrient compositions was comparable, but when spiders were fed on a prior nutrient enhanced diet, spiders killed a significantly greater number of prey over densities of prey offered. Prior diets also influenced prey choice. Taken together, these results paint this predator as an adaptive forager that makes foraging decisions based on multiple stimuli in the environment.

ADAPTIVE FORAGING IN A GENERALIST PREDATOR: IMPLICATIONS OF HABITAT STRUCTURE, DENSITY, NUTRIENTS AND PREY AVAILABILITY

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GENERAL INTRODUCTION

Food-webs are a convenient way to characterize the interactions between species. Commonly, food-webs are represented by food chains depicting the feeding relationships between predators, herbivores and primary production (Paine 1980). Food chains are known to be regulated by both bottom-up (i.e. availability of resources) and top-down (i.e. where upper trophic levels regulate the abundance of lower levels; Polis *et al.* 1989). Although numerous factors are cited to influence both bottom-up and top-down in ecological communities (i.e. Habitat structure, disturbance, nutrient levels; MacArthur and Pianka 1966; Heck and Crowder 1991; Fagan and Denno 2004), there is a growing recognition that knowledge of trophic interactions in natural communities requires incorporating foraging behavior and conditions that impact an animal's foraging behavior (see Holt & Kimbell 2007; Schmitz *et al.* 2008; Lazzaro *et al.* 2009; Abrams 2010). Foragers exhibiting plasticity in their response to environmental conditions are said to be foraging adaptively or flexible foragers (Abrams 2010). Adaptive foraging is a promising mechanistic view that has surfaced as a fusion linking food web theory with classical foraging theory, because foraging behavior is used to predict the strength of interactions between species (Beckerman 2006).

Proponents of adaptive foraging emphasize that the behavior of the predator and its potential prey can alter the strength of top-down and bottom-up forces (Beckerman et al. 2006; Schmitz et al. 2008). For example, foraging rate may change in response to the availability or profitability of prey (i.e. nutrient content or capturability) or from a patch perspective when a patch is less profitable (i.e. food availability, risk, competition) this may be a signal to move on to forage in a new location (Fretwell and Lucas 1970; Charnov 1976; Brown 1988; Laundré et al. 2001; Holt and Kimbell 2007). The most well studied attribute of foraging implemented in food web models is currently the relation of prey to predator size (Brose 2010). Results from modeling and empirical studies reviewed by Brose (2010) show that prey size alters connectance between feeding links, that is, the diet breadth of consumers is restricted by the size of prey. Predators, therefore, are not predicted to eat randomly from all available sources, and likely have the capacity to gain information about whether to forage in a patch and what to forage on given current conditions (e.g. optimal foraging theory, MacArthur and Pianka 1966; Emlen 1966). Although modeling trophic interactions using adaptive foraging is an extension of optimal foraging theory, it is more accommodating by allowing for variables other than transfer of energy to predict foraging behavior (Abrams 2010). However, at present the adaptive foraging

framework is primarily a theoretical exercise that requires further experimental tests that document a range of responses to environmental challenges.

Generalist predators are important components of many terrestrial ecosystems and have strong effects on community structure (Halaj and Wise 2000; Schmitz 2000). Thus, the foraging behavior of generalist predators continues to intrigue and challenge ecologists. Classically, these predators can influence the competition between prey species (Paine and Vadas 1969; Lubchenco 1978) by consuming the more abundant species, which allows other species in the community to grow and reproduce (Murdoch 1969). It is recognized that many systems contain generalist predators and that there are many possible interactions occurring between species that complicate predator-prey dynamics (Turchin 2003). For instance, generalist predators may consume lower trophic levels in addition to consuming each other or intraguild predation (Polis et al. 1989), which makes modeling their interactions significantly more complicated (see review Holt and Huxel 2007). Furthermore, because generalists utilize multiple prey pathways, their effect on one prey group may be weak, but preference for different groups has the potential to alter community structure (McKann and Hastings 1998; Holt and Kimbell 2007). This is of particular interest to the management of agroecosystems because the desire is to control a target pest. If predators prefer prey that is an alternative to the pest species, this may distract them from performing the desired service of pest suppression (Koss and Snyder 2005). In addition, many other factors likely influence the foraging behavior of predators. External ecological factors (i.e. habitat structure, prey availability, risk), internal condition (i.e. body condition and nutrient exposure), and density dependent interactions (i.e. interference) can influence foraging by affecting site selection, prey selection and foraging rate (see review Perry and Pianka 1997).

Spiders are an ideal group of generalist predators to use as model organisms to study foraging behavior. These predators are numerically dominant in many terrestrial ecosystems (Luczak 1979; Nyfeller and Benz 1987; Young and Edwards 1990; Nyffeler and Sunderland 2003). Being some of the most common predators, spiders have important impacts on in food webs (Halaj and Wise 2001). Because spiders achieve high densities, there is continued interest in their roles in the food web (Wise 1993; Schmitz *et al.* 2000; Finke and Denno 2006). Spiders are considered food limited and are expected to eat anything available (Riechert 1992; Bilde and Toft 1998). However, studies do show that some spiders switch between different prey pathways in relation to abundance of available prey sources (Harwood *et al.* 2004; Harwood *et al.* 2007).

In addition spiders display a wide range of behaviors that are potentially flexible and allow a spider to alter its behavior depending on the environmental conditions (Herberstein 2011).

Wolf spiders (Araneae: Lycosidae) are frequently the most abundant spiders in terrestrial ecosystems (Nyffeler and Sunderland 2003) and, in particular, spiders in the genus *Pardosa* are ubiquitous in agricultural or disturbed systems (Young and Edwards 1990; Nyffeler and Sutherland 2003; Vogel 2004; Ubick *et al.* 2005). *Pardosa milvina* is a small epigeal wolf spider that is one of the numerically dominant generalist predators in the agricultural areas of Southwestern Ohio and occurs at highly variable densities 5-100 m⁻² (Marshall *et al.* 2000). *Pardosa milvina* is an ideal candidate model organism to study adaptive foraging. Studies have documented changes in behavior to chemical information in the environment (Persons *et al.* 2001), these spiders actively choose foraging sites related to the structure of the environment and predator information (Rypstra *et al.* 2007). Understanding the importance of factors influencing their foraging behavior is important for determining the role spiders and other generalist predtors in the food web, and as insectivores in agroecosystems (Nyffeler and Sunderland 2003). Hence, more studies are needed to understand specific aspects of space use and if spiders are selective in their foraging and what factors influence selectivity.

The aim of this dissertation was to explore habitat use and prey selection in response to environment factors that may influence these foraging behaviors. I conducted four studies to explore habitat use and prey selection using a combined field and laboratory approach. *Pardosa milvina* was the focal predator used as the model generalist predator. In the first chapter, I examined how habitat structure and prey availability affects foraging site selection. Many studies of the effects of habitat structure on arthropod predators have determined that increasing the structure of areas (i.e. using mulch, or density/ diversity of plants) has positive effects on abundance and diversity (see meta-analyses Langellottto *et al.* 2004; Letourneau *et al.* 2011). However, most studies lack a control for the biological components of habitat structures that may have positive effects on bottom-up processes. I decoupled this by providing artificial structures that mimic natural structures. Interestingly, although the spider community preferred naturally structure areas, *Pardosa milvina* preferred the artificial structures where prey capture was high, structure reduced intraspecific interactions and competitors were low in abundance. In the second chapter, since I found that *Pardosa* accumulates in structured areas and interference influences their foraging, I report experiments that explicitly explore the implications of

conspecific *P. milvina* abundance and prey abundance on patch leaving and functional response. Pardosa milvina emigrated at higher rates from enclosures containing more spiders and fewer prey, and interference was found to be high in the laboratory, which lowered their functional response to prey. In the third chapter, since I found that prey abundance influences the tendency to leave a patch, I report on experiments examining the effects of prey availability on prey selection over a growing season. I collected spiders in two different agricultural field types and assayed the gut-contents of spiders to test for predation on a common prey group, Diptera, flies. Spiders exhibited variable consumption of Diptera over the season, but predation was not directly linked to abundance of prey. In the fourth chapter, I attempted to understand how prey quality impacts the feeding rate of these predators and shifts preference for prey types. Spiders are thought to be nutrient limited in the field and may select prey to alter nutrient state (Denno and Fagan 2003; Mayntz et al. 2005). However, more studies are needed to understand the effects of nutrient content on the functional relationship between predators and prey (i.e. functional response and prey choice; Abrams 2010). In performing these empirical tests and writing this dissertation, I assembled literature from a variety of disciplines and attempted to link them within the framework of adaptively foraging predators (Abrams 2010). I conclude by discussing the implication of these results for the ecology of generalist predators, and emphasize that foraging plasticity needs to be documented in order to estimate their impact on and role in ecological communities.

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CHAPTER 1

Opportunistic predator prefers habitat complexity that exposes prey while reducing cannibalism and intraguild encounters

Abstract

Structural features of habitat are known to affect the density of predators and prev and it is generally accepted that complexity provides some protection from the environment and predators, but may reduce foraging success. A next step in understanding these interactions is to decouple the impacts of both spatial and trophic ingredients of complexity to explicitly explore the trade-offs between the habitat, its effects on foraging success, and the competition that ensues as predator densities increase. I quantified the accumulation of spiders and their prey in habitat islands with different habitat complexity created in the field using natural plants, plant debris and plastic plant mimics. Spiders were observed at higher densities in complex habitat structure composed of both live plants and thatch. However, the numerically dominant predator in the system, the wolf spider Pardosa milvina, was observed at high densities in habitat islands containing plastic mimics of plants and thatch. In a laboratory experiment, I examined the interactive effects of conspecific density and habitat on the prey capture of Pardosa milvina. Thatch, with or without vertical plant structure, reduced prev capture, but the plastic fiber did not. Pair-wise interactions among spiders reduced prey capture, but this effect was moderated by thatch. Taken together, these experiments highlight the flexibility of one important predator in the food web, where multiple environmental cues intersect to explain the role of habitat complexity in determining generalist predator accumulation.

Introduction

The interactions among habitat heterogeneity and community dynamics have influenced ecological study for many years (Huffaker 1958; MacArthur and MacArthur 1961; Lawton 1983; Southwood 1996). Habitat structural complexity has substantial impacts on the abundance and diversity of animals in both aquatic (Heck and Crowder 1991; Hayse and Wissing 1996; Beck 2000; Stewart et al. 2003; Lepori et al. 2005) and terrestrial (Andow 1991; Uetz 1991; Rypstra et al. 1999; Langellotto and Denno 2004; Whittingham et al. 2006) ecosystems. Structurally diverse habitats provide a broad range of resources, insulate animals from physical disturbance, moderate environmental extremes, and provide cover for and protection from predators (Crowder and Cooper 1982; Rypstra et al. 1999; Langellotto and Denno 2004; Janssen et al. 2007). Particularly difficult to tease apart is the role of habitat complexity for interactions between predators and prey (Halaj and Wise 2002; Miyashita and Takada 2007; Birkhofer et al. 2008). In most habitats, the living and dead vegetation create complexity that are also resources for herbivores and detritivores fueling the rest of the food web (Price et al. 1980; Denno and McClure 1983; Wyman 1998; Chen and Wise 1999). In addition, habitat complexity can influence hiding, escape, and attack tactics of animals on both sides of the predator-prey equation (Crowder and Cooper 1982; Uetz 1991; Finke and Denno 2002; Legrand and Barbosa 2003; Warfe and Barmuta 2004).

For generalist predators, habitat structure can play a role in mediating any or all of the interactions occurring between individual animals including their foraging success, risk of intraguild predation, and the outcome of competitive interactions (Warfe and Barmuta 2004; Finke and Denno 2004; Grabowski *et al.* 2008). Interestingly, interactions between arthropod predators seem to be more strongly affected by structure than the consumption of prey from a different trophic level (Roda *et al.* 2000; Finke and Denno 2002; Obermaier *et al.* 2008). A recent meta-analysis concluded that habitat structure should promote persistence of predators by reducing intraguild predation (Janssen *et al.* 2007), which can have important impacts on herbivore control (Finke and Denno 2006). Structure appears to dampen the impacts of interactions between generalist predators (Finke and Denno 2004) possibly by providing escape routes for intraguild prey (Janssen *et al.* 2007). It is less clear what aspects of structure

organisms are using, and how various features of structure interact to determine the strength of interactions between foraging predators.

A recent synthesis of the information available for invertebrate natural enemies determined that the addition of detritus (e.g. leaf litter, thatch, mulch) in terrestrial systems has a more powerful effect on density than altering the plant community (Langellotto and Denno 2004). That same analysis did not uncover effects of habitat structure on the density of herbivorous prey but not all types of potential prey were incorporated into the analysis and, as aptly noted, there are cases where arthropod predators accumulate in response to high densities of detritivores (Chen and Wise 1999; McNabb *et al.* 2001; Halaj and Wise 2002; Oelbermann *et al.* 2008). Indeed, other studies have shown that enhancing the decomposer community plays a role in the establishment of robust populations of predators living up in the vegetation (Scheu 2001; Rypstra and Marshall 2005). Thus, a complete understanding of the role that habitat complexity plays in food web interactions requires further studies that uncouple structure from its trophic contribution (Bultman and Uetz 1982; Warfe and Barmuta 2004; Miyashita and Takada 2007).

In this study, I examined the avenue by which habitat complexity affected the density of spiders and prey capture of the wolf spider *Pardosa milvina* (Hentz) (Araneae, Lycosidae), which is one of the most abundant predators on the soil surface of agroecosystems in eastern North America (Young and Edwards 1990; Marshall and Rypstra 1999). I conducted an open field study that manipulated plant and soil habitat complexity using biologically based structure and physical artificial structure in a soybean agroecosystem to test the hypothesis that spiders accumulate in complex habitats independent of prey availability. In laboratory enclosures using the same biological and artificial structures, I explored the impact of habitat complexity and predator density on prey capture. I predicted that when predators reach high densities, increased interference and/or cannibalism would occur, reducing the advantages of selecting particular foraging sites. This approach enabled us to evaluate the separate and combined effects of spider density and habitat features on foraging and the frequency of cannibalism. This combination provides for a comprehensive perspective of environmental impacts on the performance of an important terrestrial generalist predator.

Materials and Methods

Study species

Wolf spiders are among the most abundant arthropod predators in agricultural systems in the United States (Nyffeler and Sunderland 2003), and as predators they may have important impacts on the food web (Carter and Rypstra 1995; Halaj et al. 2000; Scheu 2001; Halaj and Wise 2002). In Southwestern Ohio, the wolf spider Pardosa milvina (Hentz) (Araneae: Lycosidae) achieves densities of 5-100 spiders/ m^2 in soybean fields making it the numerically dominant predator for much of the season (Marshall et al. 2002). Pardosa milvina is a relatively small (20mg), vagabond species that actively pursue prev and moves between microhabitats where they accumulate in areas containing higher levels of soil substrate complexity (Marshall and Rypstra, 1999) and areas enhanced with composted material (Rypstra and Marshall 2005). An intraguild predator, the large wolf spider (100-300mg), Hogna helluo, also occurs in this system and is shown to alter habitat preferences (Buddle *et al.* 2002; Rypstra *et al.* 2007), vertical habitat use (Folz et al. 2006), foraging rate (Rypstra et al. 2007), and mating behavior (Hoefler et al. 2008) of Pardosa milvina. To further understand the subtleties of this interaction, in a field experiment, I quantified the density of Pardosa milvina, and other ground dwelling spiders such as Hogna helluo that would be intraguild competitors for space and resources. In order to link predator density to some measure of performance, I conducted two laboratory experiments, which explored the effects of habitat structure and spider density on foraging success of P. milvina.

Spider abundance and habitat complexity

To determine the effects of habitat complexity on abundance of spiders and prey availability I established replicate sets of 2 x 2 m plots in five, 0.42 ha soybean fields at Miami University's Ecology Research Center (ERC) (3 km North of Oxford, Butler County, Ohio, USA; Appendix: Plate S1). Each field measured 60 x 70 m and was separated from the surrounding habitats and one another by a 15 m border of mown grass (Kemp and Barrett 1989). The fields were tilled on 5-May-2005 and planted with rows (approximately 61 cm apart) of soybean plants (*Glycine max*, Fabaceae) on 10-May-2005. On 20-June-2005, when the soybean plants were 20 - 25 cm tall, plots were tilled to eliminate weeds, raked to smooth the soil

surface, and randomly assigned to one of six habitat complexity treatments. Four treatments (n=5) were designed as habitat features spiders would typically encounter: (1) bare soil, (2) soybeans only: two rows of plants in each plot, (3) thatch: 15-20 cm layer of wheat straw covered the soil surface, and (4) soybeans plants plus thatch: combination of treatments (1) and (3). Two additional treatments (n=5) were designed to provide similar structural complexity of the plants and thatch but lack biologically based components that would encourage growth of the prey community: (5) fiber: a 15-20 cm layer of polypropylene fiber (Textraw® Synthetic Straw, St. Simons Island, GA, USA) covered the soil surface, and (6) polyvinyl plants plus fiber: two rows of polyvinyl plants similar in structure to soybean plants combined with a 15-20 cm layer of fiber on the soil surface. To account for growth of the living soybean plants, polyvinyl stems and leaves were added to the artificial plants on 15 July 2005 to bring them to a height of 40-45 cm. On that same date, living soybean plants were trimmed so that their size matched artificial plants.

Habitat complexity of each plot was quantified on 25-July-2005 at five regularly spaced sites in each plot, one in the middle and four others approximately 15 cm from each corner. Leaf area index was used to indirectly estimate the amount of vertical vegetation in each plot by measuring transmittance of light through the vegetation using the Plant Canopy Analyzer LAI-2000 (LI-COR, INC., Lincoln, Nebraska, USA). In plots with substrate addition, thatch or fiber, depth of these structures was measured and in plots with real or artificial plants, the height and width of the plants were measured. I expected thatch and fiber to effect temperature (°C) and humidity (absolute humidity g m⁻³) at the soil surface, thus using one HOBO® data-logger (Onset Computer Corporation, Bourne, MA, USA) centered in each plot and below any thatch or fiber, I measured the mean daily temperature and humidity by logging at 15-minute intervals between 25-June-2005 and 12-July-2005 in three contrasting treatments: bare soil, soybeans plus thatch, and polyvinyl plants plus fiber.

To understand the bottom-up effects of structure on potential prey, activity density of prey was measured by placing two sticky traps on top of any soil surface structure in the plots for three days just prior to quantifying spider abundance (29-July-2005 to 2-August-2005). The traps were constructed of transparent acetate sheets measuring 8 x 24 cm. A 6.5 x 6.5 cm sampling area of the trap was coated with non-toxic adhesive (Harwood *et al.* 2004; Tangle TrapTM, Grand Rapids, MI, USA). Upon collection, traps were placed in a freezer to preserve the insects. I

counted the most common spider prey including: Collembola, Diptera, Homoptera and Orthoptera (Nentwig 1986; Nyffeler *et al.* 1994).

Spider abundance was quantified on 2-August-2005, by destructively sampling using a restricted area search (Marshall *et al.* 2000). Two steel rings (each measuring 0.75 m^2 diameter 30 cm sides) were dropped simultaneously in each plot to delineate a search region of 1.5 m^2 . One observer at each ring searched the enclosed area by systematically stirring any litter and digging up any cracks in the soil. Subsequently, plant, thatch or fiber material was sifted using a 50 mm mesh litter reducer over a white bucket to recover less obvious spiders. All spiders collected were counted and identified.

Prey capture: conspecific density and habitat complexity

Pardosa milvina used in experiments were all penultimate or adult female spiders collected between June and October 2005 from agricultural fields at Miami University's Ecology Research Center. When not involved in experiments, spiders were housed individually in plastic containers (6 cm diameter and 4 cm sides) with a 1.5 cm layer of moist soil in an environmental chamber at 25°C, 50-58% RH and 13:11 L:D cycle. To equalize hunger levels, I fed each spider two 0.32 cm Acheta domesticus (here after referred to as crickets) twice per week for at least two weeks prior to the experiment, and withheld food for seven days immediately before their scheduled test. Twenty-four hrs before the experiment commenced, individuals were marked with a drop of non-toxic paint on the dorsal surface of the abdomen to allow for identification. Just before the experiment, the carapace and abdomen width were measured to the nearest 0.1 mm using a microscope equipped with a digital micrometer. Body measurements are commonly used as indications of body condition (Anderson 1974; Jakob et al. 1996). Specifically, the carapace width is a measure of size and the abdomen of spiders is flexible and widens as the spider feeds (Jakob et al. 1996). Spiders assigned to treatments were similar in size (one-way ANOVA carapace width: $(F_{15, 202} = 0.40, P = 0.96)$ and condition (one-way ANCOVA of abdomen width using carapace as covariate; $F_{15, 202} = 1.12$, P = 0.32) prior to introduction into arenas (for data see Appendix: Table S1).

Using these laboratory standardized spiders, I conducted two experiments in order to understand the influence of predator accumulation in patches with contrasting structural features on prey capture (number of crickets captured in a 24 hr period). Experiments were conducted in

cylindrical plastic arenas (20 cm diameter with 30 cm sides) with a 4 cm layer of moist soil covering the base. The first experiment was designed to understand the impact of density and habitat structure on prey capture in all the structural treatments used in the field study. Habitat treatments (bare soil, soybean plants, thatch, soybean plants + thatch, fiber, and plastic plants + fiber) were crossed with two spider densities (one spider, and five spiders equivalent to 159 spiders m⁻²) for a minimum of 10 replicates per treatment combination. Vertical plant structure was manipulated by adding either two live soybean plants, each measuring 20-24cm in height with five leaves, or two polyvinyl plants fabricated to simulate soybean plants. To manipulate substrate structure, either a 4 cm layer of thatch or fiber covered the soil. I used 0.32 cm crickets, *Acheta domesticus*, to represent an easy to capture ground dwelling prey species. I chose crickets because they are common prey for spiders, easy to rear, and recapture following experimental manipulation. To initiate trials, 40 crickets were placed into each arena and allowed to disperse for 15 minutes before *P. milvina* were released into arenas. After a 24-hr period, the spiders were captured, and the number of crickets remaining was counted.

In the second experiment I examined the pair-wise interference between conspecifics relative to habitat structure. I used one spider density, two spiders (field density equivalent to 64 spiders m⁻²) in three representative habitat treatments (bare soil representing no structure, fiber representing artificial, and thatch representing natural structure). Habitat treatments and experimental protocols were administered in the same way as the first laboratory experiment. Prey capture from this experiment (observed prey capture when spiders forage together) was then compared to the predicted prey capture expected when spiders forage in isolation.

Statistical analyses

In the field experiment I measured the effects of habitat structure on colonizing predators. This experiment was specifically designed to address the importance of natural (biologically based) structure as compared to artificial (physical structure), and secondly to assess the importance of different structural features. Models were generated to explain predator responses (*Pardosa milvina* and other ground dwelling spider abundance) to habitat treatments in the field using Schwarz's Bayesian information criteria (BIC, Burnham and Anderson 2002). Comparison of BIC between models allows for evaluating different levels of support for the various components that could be incorporated into the model. Models are ranked according to

change in BIC (Δ BIC), and less evidence is present to support models when Δ BIC>2 (Burnham and Anderson 2002). BIC was selected because it imposes a higher penalty for extra parameters and thus identifies less complex models (Murtaugh 2009). To initiate model selection I built a model containing potential predictors that I measured in our habitat treatment plots, which included: leaf area index (LAI), prey activity density of Collembola, Diptera, Homoptera, and Orthoptera. All variables were transformed using natural log to normalize the distributions and variance except the two categorical predictor variables: habitat treatment and field (which was included as a random variable). The upper scope of model complexity contained the two-way interactions between habitat and prey groups. Model selection was conducted in R (R Development Core Team 2009) by implementing stepAIC {MASS} (Venables and Ripley 2002), which compared models using BIC (i.e. specifying k=log (n) with n= the number of cases) through a stepwise algorithm (Venables and Ripley 2002).

Since I was specifically interested in the impact of our habitat manipulations on spider abundance, I estimated the effect size for each of our habitat treatments on the abundance of *Pardosa milvina* and other spiders. I calculated Hedge's unbiased g^* effect size by computing the mean difference and 95% confidence intervals between the natural log transformed abundance observed in each of the habitat treatments and bare soil containing no additional habitat treatment (Nakagawa and Cuthill 2006). Because many of the bare soil plots contained few if any spiders, I used the standard deviation of the habitat treatment instead of the pooled standard deviation.

In the first laboratory experiment, I measured the effects of habitat complexity and predator density on prey capture (number of crickets captured in a 24 hr period). Weighted least squares two-way ANOVA was used to test for the main effects of habitat (all habitat treatments) and *P. milvina* density (one vs. five) on prey capture (number of crickets captured in a 24 hr period). Significant main effects were compared using the post-hoc Tukey (HSD) multiple comparisons. Prior to analysis, prey capture was natural log transformed to normalize distributions and variance. Since *P. milvina* are cannibalistic (Buddle *et al.* 2003; Rypstra and Samu 2005), logistic regression was used to test for the effect of habitat treatments on the frequency of cannibalism. Because these were sealed laboratory arenas, any spider missing at the end of the 24 hr period was counted as one cannibalism event.

In a second experiment I measured prey capture of two *P. milvina* in an arena to assess interference between individuals as related to habitat type. To test for interference between individuals, I compared the observed total number of crickets captured over a 24 hr period with predictions from a basic rule of probability used to test for interference (Soluk and Collins 1988; Sih 1998). The probability that crickets (prey) were captured by two *P. milvina* foraging in isolation equals one minus the probability that crickets were not captured. Rearrangement of this expression, taking into consideration independence between each predator's capture, and multiplying this probability by the initial number of prey equals the predicted prey capture (C):

$$C = Np (P_a + P_b - P_a * P_b)$$

Where (Np) equals the initial prey density, (P_a and P_b) equal the probability of crickets captured by two *P. milvina* foraging in isolation, and (P*P) term equals the probability that prey were not captured by either of the spiders (Soluk and Collins 1988; Sih 1998). To test the null hypothesis that observed equaled predicted and was consistent across habitat type, I used a two-way ANOVA relating cricket capture to habitat type and an indicator variable (state) to test for differences between predicted and observed at each habitat level. When observed is greater than predicted this indicates synergistic capture or kill rates of prey, however when observed is less than predicted this indicates that predators interfere with each other, which would reduce capture rates. A significant interaction between the indicator and habitat would reject the null hypothesis that interference levels are equal in habitat types tested. These analyses were conducted in JMP 8 (SAS Institute, Inc).

Results

Spider abundance and habitat complexity

Although soil surface structure was equivalent between natural and artificial plots, there was more vertical vegetation (soybean plants) in the natural plots as compared to the artificial plots (Appendix: Table S2). The amount of surface structure, depth of either thatch or fiber, was similar across treatments (ANOVA $F_{3,72} = 0.65$, P = 0.59), but by the end of the experiment the soybean plants (height=72.65±8.66 cm) were significantly larger than the polyvinyl plants (height = 39.2±2.75cm) (ANOVA $F_{1,34}$ = 464.73, P < 0.0001) despite of our efforts to equalize them. Correspondingly, our measurements Leaf Area Index were greater in plots containing soybeans than polyvinyl plants ($F_{1,34}$ = 32.05, P < 0.0001).

The microclimate was similar between the natural and artificially structured plots, but more variable in the plots lacking structure than either of the structured plots (Appendix: Table S2). Bare soil plots had the lowest absolute relative humidity (abs humidity = 15.92 g m⁻³; $F_{2,269}$ = 7.49, P = 0.0007) and the highest mean daily temperature (25.26°C; $F_{2,269} = 7.69$, P = 0.0006) and the fluctuation in temperature (CV = 10.13) and humidity (CV = 32.29) was highest in the no structure plots. The artificial and natural structured plots were similar in mean daily humidity, but there was more variability in humidity in the artificial (CV = 23.99) as compared to the natural (CV = 16.13). Neither temperature nor fluctuation in temperature differed between artificial and natural plots.

Total prey availability was highest in plots containing thatch or living soybean plants and thatch, and this pattern was largely driven by the abundance of Collembola, a common prey group for ground dwelling spiders ($F_{5, 29} = 10.77$, p < 0.0001, Fig. 1a). Contrastingly, the structure provided by the fiber with or without the polyvinyl plants did not enhance total prey availability as compared to bare soil or soybean plants alone.

Pardosa milvina was the numerically dominant spider in our community comprising 68.3% of the 389 spiders captured. Clearly the ground dwelling spider community (Appendix: Table S3) and the *P. milvina* increased in density when habitat structure was present in experimental plots (Fig. 1b). Model selection determined that models containing habitat structure and field provide the most evidence of support for predicting the abundance response of *P. milvina* (Table 1). Competing models containing prey availability covariates all resulted in Δ BIC > 2 for *P. milvina*, which indicates that there was less evidence in the data supporting models containing prey covariates, and models with only prey availability were inferior by comparison and resulted in substantial Δ BIC. Conversely, for the other ground dwelling spiders, models lacking prey availability covariates (Table 1, Fig. 1b). Specifically, the top competing models suggest that some combination of habitat structure and the abundance of Diptera, Homoptera and Collembola provide better insight into habitat associations for the rest of the ground dwelling spider community, as compared to habitat structure alone.

The effect of habitat treatments on all spiders was least when only soybean plants were added to bare soil, and addition of soil substrate complexity had strong effects where abundance was highest in treatments with vertical plants or plant like structure added in combination with

thatch (Table 2, Fig. 1b). *Pardosa milvina* responded differentially to both the position of the structure (plant like or soil surface) and to the nature of structure provided (natural or artificial). Natural or artificial structure had similar effects on the abundance of *Pardosa milvina*. Interestingly, although no *P. milvina* were found in patches with soybeans alone, combining the vertical structure of soybean plants or plastic plants with thatch or fiber had the largest positive effect on abundance. The other ground dwellings spiders, conversely, primarily responded positively to the natural habitat treatments (Table 2, Fig. 1b). These spiders were lowest in abundance in plots containing artificial structure, and the largest effects were observed when thatch was paired with soybean plants, which was also the treatment containing the highest overall prey abundance (Table 2, Fig. 1).

Prey capture: conspecific density and habitat complexity

Prey capture was consistently higher at both densities in arenas containing no structure, soybean plants or artificial structure as compared to the natural structure ($F_{16, 179} = 60.57$, P < 0.0001; Fig. 2). The presence of thatch generally reduced prey capture with or without the addition of soybean plants. Cannibalism was only observed in the natural structure treatments at high-density treatments (Fig. 3). At high-density, the likelihood that cannibalism occurred was significantly reduced by the presence of thatch (Logistic regression, $\chi^2 = 8.38$, P = 0.0038) and there was no interaction between the presence of plants and the presence of thatch on the occurrence of cannibalism (Logistic regression, $\chi^2 = 2.11$, P = 0.15; Fig. 3).

To assess interference between individual predators, I compared observed when spiders were combined to predicted prey capture in isolation. The observed number of prey captured when two *P.milvina* were foraging in bare soil or the artificial fiber was significantly lower than the predicted in each of these habitat types (Table 3, Fig. 4). This indicates that in bare soil or fiber, the presence of a conspecific lowered their combined capture rate. Conversely, prey capture in the thatch by two *P. milvina* was similar to the predicted consumption, which indicates that adding this form of structure reduced interactions between conspecifics. There was a significant interaction term indicating that interference in the thatch habitat types, and this can be explained be a reduction in interference in the thatch habitat leading to near equivalence between predicted and observed.

Discussion

Members of the genus *Pardosa* are highly successful in agroecosystems worldwide (Young and Edwards 1990; Nyffeler and Sutherland 2003; Vogel 2004; Ubick *et al.* 2005) and have direct consumptive effects in food webs (Gratton and Denno 2003; Wise 2004) as well as nonlethal effects (Hlivko and Rypstra 2003). Data from the current study suggest that these ubiquitous predators actively colonize novel unoccupied habitat patches based on prey capture and on avoidance of competitors or intraguild predators, providing insight into the success of these generalist predators in highly disturbed habitats.

Our study is one of few that have separated habitat structure as a spatial resource (i.e. increased living space, Bell *et al.* 1991) and as a trophic resource (i.e. increased supplemental resources for predators and food provisions for potential prey, Miyashita and Takada 2007; Birkhofer *et al.* 2008). Our approach also was unique by combining more than one habitat structural type (i.e. vertical plant structure and soil surface structure) and using artificial representatives to separate spiders' response to structure from that of biologically-based resources encouraging recruitment of potential prey. In addition, laboratory trials enabled us to comprehensively explore effects of structure and the resultant increase in predator density on the foraging performance of the most common species in our system.

I anticipated that habitat structure would increase the density of arthropod predators, as many studies have documented such a correlation (Halaj *et al.* 2000; Langellotto and Denno 2004). The benefits, however, of different habitat features to generalist predators remains poorly understood because multiple, possibly competing mechanisms help explain abundance and diversity patterns (Denno *et al.* 2005; Janssen *et al.* 2007; Schmitz 2008). Here I show by selecting structurally complex habitat patches the wolf spider, *Pardosa milvina*, is able to capture prey and avoid intraguild predation. Surprisingly, even though the activity density of prey appeared to be important to other spider species in our field study, I found no evidence that the absolute availability of prey was important for this *Pardosa. Pardosa milvina* accumulated in plastic mulch in field plots with low prey abundance and low densities of other spiders. Prey capture in the laboratory was reduced in straw thatch structurally similar to the plastic fiber, but there was no evidence that the plastic fiber interfered with prey capture efficiency. Although I found that high spider densities reached in the plastic fiber would likely lead to higher levels of intraspecific competition than in the straw thatch habitat, cannibalism was eliminated.

Taken together these experiments suggest that habitat selection in *P. milvina* is motivated by their ability to forage on and within certain substrate structures more than the prey available in a specific habitat patch. Of all the factors I measured, habitat complexity was the most important predictor of the overall spider density in our field plots. Interestingly, although prey availability was important in explaining the abundance of other spiders, the abundance of most common spider species, *P. milvina*, was linked more closely to habitat structure. This result suggests that cues used by spiders to select foraging sites vary between species, and although the spider community in our agroecosystem appeared to respond to increases in their trophic resources, Pardosa milvina did not. Synthesis of ecological studies on spiders indicate that, as a group, spiders are food limited in nature and prey consumption and foraging site selection has strong effects on fitness (Wise 1993; Halaj and Wise 2002; Wise 2006) and so I expected the impact of structure and prey abundance to be linked as they were for the non-P. milvina component of the spider community in this study. In fact, in a previous study of *P. milvina* conducted at the same field site, their densities increased in plots where prey availability was augmented through addition of detritus on the soil surface (Marshall et al. 2000). Although the results of this study are consistent with the generalization that *P. milvina* is successful in highly disturbed agroecosystems because it is a good colonizer, these results also suggest that specific habitat selection mechanisms in this generalist predator is not as simple as it has seemed from previous work (Marshall et al. 2000, 2006; Buddle et al. 2003).

Pardosa milvina accumulated in areas where cannibalism was low (Fig. 3), the abundance of competitors or intraguild predators was low (Fig. 1), and their predation rate was high (Fig. 2). In our laboratory experiment, I found that even at high densities of *P. milvina*, increased habitat complexity reduced predation rate. High spider density led to prey depletion in experimental arenas and the likelihood of cannibalism was reduced when thatch was present. However, interactions between *P. milvina* foraging together in the laboratory reduced predation rate (Fig. 4). Langellotto and Denno (2006) determined that thatch reduced cannibalism in *Pardosa littoralis* when lower levels of prey were available. In our study, cannibalism occurred only in treatments containing high densities of *P. milvina* and more frequently when no substrate structure was present. Notably, these were treatments where few prey were remaining at the end of the trial.

In an open field setting, intraspecific interference may be mitigated by the versatility of *P. milvina* to make broad use of available habitat patches (Marshall and Rypstra 1999; Buddle *et al.* 2003), which is consistent with the idea that scaling of interaction strength is an important component of predator space use (Drapela *et al.* 2008). For instance, a congener occupying agroecosystems of eastern Europe has been characterized as a "sit and move" predator that changes foraging location frequently (Samu *et al.* 2003), which is likely the reason that populations of *P. milvina* are not sensitive to habitat fragmentation or to the destruction of neighboring patches of habitat (Marshall *et al.* 2006). Thus, the intraspecific interference and cannibalism I observed at increasing densities in laboratory experiments, where neither predator nor prey could escape, are probably artifacts of the experiment and an overestimate. In a more natural situation, they can more easily adjust to the presence of a conspecific by relocating.

Not surprisingly, the thatch and fiber mulches provided protection to the spiders from the microclimate fluctuations that occur in exposed plots of soil during an Ohio summer. Indeed, it has been demonstrated that the distribution and abundance of other spiders are influenced by their ability to withstand temperature and humidity extremes (Henschel *et al.* 1992; DeVito *et al.* 2004). Our field study revealed that natural wheat straw with live soybean plants moderated microclimatic extremes to a greater degree than the plastic mimics, but *P. milvina* selected artificially structured patches anyway. Although temperature and humidity certainly would affect habitat selection in these small ectotherms, *P. milvina* seemed to compromise by selecting an environment more moderate than bare soil but also where prey-capture efficiency was high and the threat of competitors or predators was low.

I initially assumed that additional habitat structure would hinder foraging ability, as such a connection has been documented in a wide variety of arthropod natural enemy taxa (Andow and Prokym 1990; Lukianchuk and Smith 1997; Clark *et al.* 1999; Legand and Barbosa 2003). In the field, addition of either live or artificial plants increased the density of *P. milvina* and in the laboratory, *P. milvina* frequently used plants as resting sites. However, the presence of plants had little impact on prey capture in the laboratory (Fig. 2). It is possible, that in the field, plants provided critical protection from temperature extremes, however temperature and humidity differences between plots with and without plants was not quantified. Nonetheless, the finding that *P. milvina* preferred the plastic patches with more humidity fluctuation and slightly higher temperatures than the thatch and live soybean treatments indicates that subtle differences in

microclimate provided by the plants were not driving this preference. Alternatively, *P. milvina* shifted their habitat use perhaps due to the presence of chemical information from the larger syntopic intraguild predator, *Hogna helluo* (Araneae: Lycosidae) (Rypstra *et al.* 2007). Folz *et al.* (2006) documented that *P. milvina* climb up on vertical surfaces to avoid exposure to *H. helluo* chemical cues, placing them out of the predatory reach of this larger, slower wolf spider. Therefore, it is possible that *P. milvina* selects a habitat containing plants, which provide escapes routes for this small agile spider. This response would account for the observed difference in *P. milvina* density between plots with natural or artificial mulch on the soil surface and those with mulch and live or artificial plants.

Foraging site selection appears to be dynamic in *P. milvina*; lower predation risk coupled with greater capture efficiency may explain why P. milvina chose novel habitats containing artificial structure over natural ones preferred by other spiders. I was surprised to discover that this spider was so effective at prey capture in plastic fiber habitats. Wolf spiders have scopulae, a dense tuft of bristles under their tarsal claws, that enables them to climb burnished surfaces (Foelix 1996). These structures may have allowed *P. milvina* to gain more traction than the cricket prey on the plastic fiber and thus enhanced their prey capture success. Whatever the reason, the habitat and foraging differences between P. milvina, and the rest of the spider community offers one explanation for our failure to detect strong top-down effects by arthropod predators in terrestrial systems (Halaj and Wise 2001). Early work suggested that spider assemblages were more apt to provide effective biological control than a few species (Riechert and Lockley 1984). More recently, a meta-analysis showed that overall natural enemy diversity improves pest regulation (Letourneau et al. 2009); however, agricultural intensification that reduces both within field and landscape complexity, negatively impacts natural enemy abundance and diversity (Tscharntke et al. 2007). In our system the numerically dominant arthropod predator responded differently to habitat structural features when compared to the rest of the spider community. As these predators sort themselves out in space they may enhance their overall impact on plant production through niche partitioning (Finke and Snyder 2008), but a complex suite of direct, and indirect behavioral responses to habitat, other predators, prey, and conspecifics make those effects more difficult to detect. These results, therefore, underscore the importance of continued efforts to characterize important resources required to promote and maintain high abundance and diversity of predators in managed systems.

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Table 1. Comparison of models examining the influence of habitat structure plots (Habitat), prey availability (Coll=Collembola, Dipt=Diptera, Hom=Homoptera, Orth=Orthoptera), Leaf area index (LAI), and field (a random variable), on the abundance of *Pardosa milvina* and other ground dwelling spiders. Models were ranked by BIC values, Schwarz's Bayesian information criteria, and less evidence is present to support models when Δ BIC>2.

Models	BIC	ΔΒΙϹ
Pardosa milvina		
Habitat + Field	-62.40	0.00
Habitat + Field + Orth	-60.31	2.09
Habitat + Field + Orth + Hom	-57.46	4.94
Habitat + Field + Orth+ Hom +Dipt	-57.29	5.12
Habitat + Field + Orth + Hom + Dipt + LAI	-54.35	8.05
Habitat + Field + Coll +Dipt + Hom+ Orth + LAI	-47.75	14.65
Habitat	-46.55	15.85
Orth	15.44	77.84
Field	15.79	78.19
Coll+ Dipt + Orth + LAI	18.64	81.04
Coll + Dipt + Hom + Orth	25.25	87.65
Other spiders		
Habitat + Dipt + Hom	-27.40	0.00
Habitat + Dipt	-26.96	0.44
Habitat + Dipt + Coll	-26.78	0.62
Habitat + Dipt + Coll+ LAI	-24.66	2.74
Habitat + Dipt + Coll + LAI + Field	-24.56	2.84
Habitat + Dipt + Coll+ Orth + LAI + Field	-24.12	3.28
Habitat + Dipt + Orth	-23.74	3.66
Habitat + Dipt + Orth + Field	-23.68	3.72
Habitat + Dipt+ Orth + Field + LAI	-23.43	3.97
Habitat	-21.45	5.95
Habitat + Dipt + Field + LAI	-20.40	7.01
Habitat + Orth + Field + LAI	-15.43	11.97
Orth + Field + LAI	-6.21	21.19

Table 2. Analysis of effect size of habitat treatments on the abundance of *Pardosa milvina*, and other ground dwelling spiders. Values for effect size represent Hedge's unbiased g^* and values in parentheses represent 95% confidence intervals.

Habitat treatment	Pardosa milvina	Other spiders
Soybean plants	0.66(0.74)	1.57(1.58)
Thatch	4.85(0.84)	3.96(1.14)
Soybean plants + Thatch	5.39(0.88)	5.41(1.12)
Fiber	5.27(0.65)	2.23(1.18)
Polyvinyl plants + Fiber	9.69(0.56)	2.63(0.90)

Table 3. Two-way ANOVA of the effects of habitat on prey capture by *Pardosa milvina* to assess interference in different habitats in a laboratory experiment. The prey capture of two spiders foraging together was compared to the predicted prey capture (Soluk and Collins 1988, Sih 1998) of two spiders foraging in isolation within three different habitat types. State represents an indicator variable of either observed or predicted prey capture. Bonferroni adjusted orthogonal linear contrasts were used in hypothesis testing for the interaction between Habitat treatment and State (α =0.05/3=0.017).

Source	df	SS	F	Р
State	1	460.93	58.46	< 0.0001
Habitat treatment	2	958.51	60.79	< 0.0001
Habitat treatment x State (interaction)	2	58.09	3.68	0.0316
Observed Bare vs. Predicted Bare	1	274.51	34.82	< 0.0001
Observed Fiber vs. Predicted Fiber	1	204.64	25.96	< 0.0001
Observed Thatch vs. Predicted Thatch	1	39.83	5.05	0.0287
Residual	54	425.74		

Figure 1. Effect of habitat treatments on abundance (mean \pm SE) of (a) prey activity density (no. 42.25 cm²), and (b) *Pardosa milvina* and other spiders in response to habitat treatments in a soybean agroecosystem (n=5). Natural and artificial represent the complexity treatments compared in the analyses.



Figure 2. Interaction plots showing results from a laboratory predation experiment measuring the prey capture (crickets captured mean \pm SE over a 24 hr period) by *Pardosa milvina* exposed to a factorial design containing the habitat treatments listed in the legend and two density levels (one spider per arena, five spiders per arena of field density of 159 spiders m⁻²). Asterisk indicates significant differences determined by post-hoc tests (Tukey-Kramer, *P*<0.05).



Pardosa milvina density

Figure 3. Proportion of laboratory trials where cannibalism was observed in the wolf spider, *Pardosa milvina*. Cannibalism was only observed in the five spider treatment (field density of 159 spiders m⁻²). If cannibalism was observed, it was only observed once in an experimental trial.



Figure 4. Interaction plots comparing the observed and predicted prey capture (crickets captured mean±SE over a 24 hr period) by *Pardosa milvina* in isolation and when together. The one spider data were displayed for reference of one spider foraging in isolation, and symbol for two spiders represents the observed prey capture when two *P. milvina* were foraging in a habitat together. The predicted two spider values were obtained by combining the prey capture probabilities of one *P. milvina* in isolation (see text and Soluk and Collins 1988; Sih 1998).



Appendix

Plate S1. Aerial photograph of the experimental soybean agroecosystem at Miami University's Ecology Research Center, Butler County, Oxford, OH. The field site contains 12 soybean fields measuring 60×70 m (a). Photographs of the habitat islands containing (b) natural and (c) artificial treatments containing both plant structure and soil surface structure additions.

(a)



(b)





Table S1. Mean size (carapace width) ± 1 SE and mean abdomen width ± 1 SE of *Pardosa milvina* before treatments. Treatments represent density (one for one spider, two for two spiders, five for five spiders added to arenas), and in parentheses the habitat that spiders were assigned to for the experiments. These data were used to confirm that spiders were similar in size and condition prior to treatment using ANCOVA with the carapace width as the covariate of abdomen.

	Carapace width before	Abdomen width before
Ireatment	(mm)	(mm)
Experiment 1		
One (Bare soil)	2.21±0.17	1.94 ± 0.31
One (Soybean plants)	2.18±0.19	1.97 ± 0.24
One (Thatch)	2.19±0.18	1.97 ± 0.23
One (Soy + Thatch)	2.18±0.22	1.96 ± 0.26
One (Fiber)	2.11±0.11	1.99 ± 0.24
One (Polyvinyl plants + fiber)	2.20±0.14	1.98 ± 0.15
Five (Bare soil)	2.23±0.17	2.01 ± 0.32
Five (Soybean plants)	2.24 ± 0.20	2.03 ± 0.27
Five (Thatch)	2.22±0.19	2.03 ± 0.34
Five (Soy + Thatch)	2.18±0.21	2.05 ± 0.31
Five (Fiber)	2.25±0.13	2.01 ± 0.21
Five (Polyvinyl plants + Fiber)	2.26±0.11	$2.00{\pm}0.22$
Experiment 2		
Two (Bare soil)	2.16±0.19	1.89 ± 0.20
Two (Fiber)	2.29±0.12	2.11±0.19
Two (Thatch)	2.18±0.14	1.92±0.29

Table S2. Habitat structure and microclimate measurements of experimental field plots. Measurements represent the quantity of habitat structure of each plot including: plant size, depth of substrate and leaf area index (LAI). Temperature and relative humidity data are daily averages, where I calculated the daily mean temperature and humidity measured by our data loggers within plots over a 25-day period. Coefficient of variation (CV) represents the daily fluctuation in temperature and humidity. Superscripts represent Tukey pair-wise comparisons with dissimilar letters representing significant treatment differences (p<0.05). All values represent mean ± 1 SE.

	Natural treatments				Artificial treatments		
Measurements	Bare soil	Soybean plants (Soy)	Wheat straw (Thatch)	Soy + Thatch	Fiber	Plastic plants + fiber	
Habitat structure							
Plants per plot		60.4±2.3		52.8±6.9		50	
Plant height (cm)		$69.3{\pm}5.8^{a}$		72.7 ± 8.7^{a}		39.2 ± 2.8^{b}	
Plant width (cm)		49.2±3.6 ^a		$49.2{\pm}5.4^{a}$		24.4±3.3 ^a	
(cm)			7.52±1.9 ^{ns}	7.08±1.8 ^{ns}	7.87±2.8 ^{ns}	7.88±1.4 ^{ns}	
Leaf area index	1.56±0.7 ^b	2.44±0.6 ^a	1.15±0.3 ^b	$2.78{\pm}0.4^{a}$	$1.30{\pm}0.4^{b}$	1.48±0.3 ^b	
Microclimate							
Mean daily temperature (°C)	25.26 ^b			23.94 ^a		24.62 ^{ab}	
CV of temperature	10.13			8.28		8.93	
Mean daily							
(g/m^3)	15.92 ^b			18.29 ^a		17.56 ^a	
humidity	32.29			16.13		23.99	

Table S3. Mean abundance of other spiders sampled in experimental plots at the Ecology Research Center, ERC. Spiders in bold are either known or potential competitors with *Pardosa milvina* as they utilize similar foraging tactics and live on the soil surface.

			Habitat treatment					
Genus	Family	Feeding guild*	Bare soil	Soy	Thatch	Soy + Thatch	Fiber	Plastic plants+ Fiber
Hogna helluo	Lycosidae	Hunting ground runner Hunting	0	0	15	13	4	3
Pirata sp.	Lycosidae	ground runner Hunting	0	0	4	18	0	0
Rabidosa sp.	Lycosidae	ground runner Hunting	0	0	0	2	0	2
Schizocosa sn.	Lycosidae	ground runner	0	0	0	0	1	0
Xysticus sp.	Thomisidae	Hunting ambush	0	1	0	0	0	1

*Spider guilds proposed by Uetz *et al.* (1999) through use of hierarchical cluster analysis to differentiate groups based on web use, foraging mode, web type, and microhabitat use.

CHAPTER 2

Prey availability and mutual interference influence foraging behavior in a generalist arthropod predator

Abstract

Interactions between predators foraging in the same patch can have substantial impacts on patch use and functional response. Theoretical studies have yet to resolve a universal form of the functional response, and changes in the feeding rate of predators likely have important implications for their distribution patterns. I investigated the tendency of the hunting spider, Pardosa milvina, to remain in a patch in response to manipulations of prev and predator abundance. Proportion of spiders leaving patches was highest when prey was manipulated to represent a prey depleted area. Although augmenting enclosures with seven times the natural density of prey significantly decreased patch-leaving rate, a greater proportion of spiders left enclosures containing higher predator abundance. To estimate the magnitude of interference between predators influencing feeding rate in areas varying in predator and prey abundance, I conducted a functional response experiment in the laboratory, and fit competing functional response models. All models converged on non-zero estimates of the predator interference parameter. Furthermore, the Hassell-Varely model was the best fitting model with a value of the interference greater than one. These results suggest that relative abundance of prey to predators potentially sets an interference threshold, at which point these predators search for a new foraging location.

Introduction

At the cutting edge of understanding the functioning of ecological communities is the integration of adaptive foraging by predators and prey (Abrams 2010; Beckerman et al. 2010). Within this framework, the behavior of individuals, populations, or communities influences the dynamics of food webs. With origins in the "Ideal Free Distribution", predators foraging adaptively over the landscape attempt to maximize intake rate on prey by moving to high quality foraging sites that increase encounter rates with prey (Fretwell and Lucus 1970; McNamara and Houston 1990; Brown and Kotler 2004; Abrams 2007). Furthermore, local biotic and abiotic factors influence the quality of a patch by altering interactions between animals, and by moderating environmental conditions (Hassell and May 1973; Sih 1984; Stephens and Krebs 1986; Frexwell et al. 1999; Rypstra et al. 1999; Langellotto and Denno 2004; Janssen et al. 2007; Schmidt and Rypstra 2010). For instance, areas with high prey abundance would be areas where predators can capture more prey over a given amount of time, which would lead to greater payoffs over time than areas with low prey availability (Charnov et al. 1976; Iwasa et al. 1981). Conversely, factors that lower the quality of a foraging site are interactions between conspecifics that lower attack rate on prey (mutual interference; Hassell and Varley 1969) or risk of predation within or between patches (landscape of fear; Laundre' et al. 2001). Thus, the quality of a foraging site is determined by food availability, risk of mortality, and intraspecific competition (reviewed by Perry and Pianka 1999).

One way to capture how predators are foraging is by measuring the effects of different factors on the functional response. Predators, in particular, are expected to move between patches to select foraging sites containing high prey abundance, which would increase intake rate (Ford 1978; Abrams 2007). From the classic prey dependent perspective, prey density alone influences the feeding rate of predators (Solomon 1949; Holling 1959). Specifically, attack efficiency increases with prey abundance to a saturation point where handling time of prey slows the feeding rate generating an asymptotic relationship between prey abundance and feeding rate. Because predators aggregate in areas of high prey abundance, predator-predator encounters are likely to decrease the feeding rate on prey (Hassell 1978). The best form of the functional response is widely debated (Abrams and Ginzburg 2000; Skalski and Gulliam 2001; Jensen and Ginzburg 2005); therefore, further studies are needed to understand how changes in feeding rate influence the distribution patterns of predators (van der Meer and Ens 1997; Anderson 2010).

Predator dependent forms of functional response model the effects of predator abundance in addition to prey abundance on feeding rates (Hassell and Varley 1969; Beddington 1975; DeAngelis et al. 1975; Arditi and Saïah 1992; van der Meer and Ens 1997; Abrams and Ginzburg 2000). The difference between these models and the classic prey dependent functional forms is that an extra parameter is added to account for the negative effects of predators wasting time, interference, in aggressive or other activities when they bump into each other. Interference is predicted to be induced by increased abundance of predators, which would lower the maximum asymptotic feeding rate on prey. There are several competing mechanistic models: the Beddington-DeAngelis (1975), where interference between predators is predicted to matter only when prey availability is low resulting in a reduction in the area a predator can search in a given amount of time; Crowley-Martin (1989), which is similar to the Beddington DeAngelis (1975), interference is predicted to be important at all predator densities, and inference is predicted to interrupt feeding, so feeding time is restricted by interference; and finally, predator dependence can also be modeled by estimating the effects of predator abundance on attack rate, and in the special ratio-dependent case, feeding rate is dependent on the ratio of prey to predators not the abundance of each (Hassell and Varley 1969; Arditi and Ginzburg 1989; Arditi and Akcakaya 1990). Further empirical tests are needed to establish which systems behave consistent with which type of predator dependent models (Slobodkin 1992; Skalaki and Gilliam 2001), and determine the relationship between patch use and functional response (Anderson 2010). Here I report on an empirical study that investigates both of these aspects of foraging using a combined field and laboratory approach.

Schmidt and Rypstra (2010, Chapter 1) found evidence for interference between individual wolf spiders, *Pardosa milvina* Hentz (Araneae: Lycosidae), which was mediated by habitat structure or complexity. Here I report a follow up study designed to explore the effects of prey and predator abundance on interference between these predators. In the field study I used the likelihood of leaving a patch and a measure of consumption as evidence of interference. In a second experiment, I manipulated predator and prey abundance to evaluate functional response models, and determine if interference was important when predator foraging area was held constant over a range of predator and prey abundances. I compared competing functional response models (Hassell-Varley 1969; Beddington-DeAngelis 1975; Crowley-Martin 1989) to select among alternative models of prey capture rates and predator interferences. By using this

combined approach I was able to study the influence of density dependence on this predator by linking feeding rate to dispersal.

Materials and methods

All spiders used in experiments were adult female Pardosa milvina collected from the Ecology Research Center, Butler County, Ohio (referred to as ERC). Spiders were maintained within separate containers in an environmental chamber (13 hrs light: 11 dark, 60% RH, 25°C). This wolf spider is numerically dominant predator in riparian and agroecosystems in eastern North America (Dondale and Redner 1990; Young and Edwards 1990). These spiders reach highly variable densities in soybean fields ranging from 5-100 m⁻² (Marshall et al. 2002), and density is observed to be driven by a combination of prey availability (Marshall et al. 2000), landscape pattern (Marshall and Rypstra 2006), and habitat complexity (Schmidt and Rypstra 2010, Chapter 1). Their variable densities are likely a result of their sit-and-pursue foraging strategy, where they move between quality foraging sites (Nyffeler et al. 1994; Samu et al. 2003). Foraging site selection is related to the presence of intraguild predators and type of habitat present (Rypstra et al. 2007), and the rate at which a single individual forages in isolation, or their functional response, is characterized as a Holling type II (Walker and Rypstra 2002; and Chapter 4). There is also evidence that the tendency to leave a patch is potentially driven by habitat quality (i.e. presence of habitat structure) and density of conspecifics (Buddle et al. 2003a).

Interference and patch leaving

I tested for the influence of prey availability and predator abundance on patch leaving of the wolf spider, *Pardosa milvina*. Patch leaving was measured from enclosures established in the soybean fields at the ERC. Enclosures were constructed of sheet metal 30 cm in height to form a 0.75 m² circular area with 10 cm buried in the ground. To prevent predators and prey from climbing the walls of the enclosures, the inside wall of each enclosure was cleaned and coated with a silicone spray. To prevent escape, the top of each enclosure was covered with 50 mm mesh. Each enclosure had four 0.64 cm holes drilled into the sides (opposing sides, evenly spaced) at the soil surface level. Plastic cups (0.47 L), with the same sized drill holes at 5 cm below the lid of the cup, were attached to the side of the enclosure so that spiders leaving the

enclosures were captured in the cups. Each cup contained a small piece of crumbled up paper towel provide structure in the cup as a means to provide refuge from sun and prevent cannibalism. Each plastic pitfall cups also had a lid to prevent escape. This design allows introduced spiders the choice to leave enclosures through the holes provided (Buddle *et al.* 2003b). Enclosures were placed a minimum of 10 m apart in one of four 60 x 70 m soybean fields. Once enclosures were positioned in the field, we removed as many prey as possible by suction sampling the soil surface within enclosures multiple times using a Ryobi® garden vacuum (Techtronic Industries Co. North America) until no prey were detected (*as in* Snyder *et al.* 2003). Following suction sampling, all enclosures received 5 cm layer of wheat straw thatch that evenly covered the bottom of each enclosure to provide structural complexity.

Eight replicates of each prey treatment (removed or augmented) and predator treatment (low 7 = spiders and high =28 spiders) combination were conducted. Prey availability was enhanced by adding seven suction samples to each prey enhanced treatments (Appendix: Table 1). Suction samples were taken using the garden vacuum, fitted with an insect net to capture prey. I suction sampled within a 1 m² metal ring for 20 seconds from randomly chosen locations in the grassy border adjacent to the field. Each sample was emptied into a bucket, and all spiders and predators were removed by hand using forceps. Following sorting, each sample was immediately transferred to the respective enclosure and the enclosure was again covered with the 50 mm mesh. Prey were given a 30 minute period of dispersal within the enclosure.

Predators, female *Pardosa milvina*, were hand collected from the soybean fields at the ERC. Each spider was collected in separate a 10 ml plastic vial containing a moist paper towel (to keep them from drying out), and kept in the environmental chamber overnight without food. Two hrs before releasing the spiders in field enclosures, I measured the cephalothorax width, and the abdomen width of seven spiders from each treatment group using a digital micrometer attached to a stereomicroscope (accuracy of 0.01 mm). The abdomen width expands as a spider consumes prey and so changes in abdomen width reflect recent feeding history, while the carapace width does not change except at molt and so provides a measure of absolute size (see Rypstra *et al.* 2007).

To initiate the experiment, I opened the side of the mesh cover and carefully added the required spiders for each predator treatment. To reduce interactions between spiders, I deliberately released spiders individually in different locations within the enclosure. I returned

to the enclosures 24 hrs later to collect spiders from the cups designed to catch any spiders leaving, and from within enclosures. I thoroughly inspected the straw and within any cracks in the soil surface for spiders remaining in the enclosures. To measure prey consumption during the 24 hr period, I randomly selected 5-7 spiders from each treatment and measured their abdomen width (i.e. to calculate consumption as change in abdomen over the 24 hr period).

Interference and feeding rate

To test for the effects of prey and predator abundance on the feeding rate of female P. milvina, I conducted a functional response experiment in the laboratory. In a factorial design I crossed four abundance levels of *P. milvina* (1, 2, 4, 8) with three prey abundance, 0.32 cm crickets, Acheta domesticus (40, 80, 160), for a total of 12 treatment combinations each replicated eight times. Female *P. milvina* were used because their functional response is influenced by prey abundance (Walker and Rypstra 2002). To standardize the diet of the spiders, I fed each spider two 0.32 cm crickets, twice per week for a minimum of three weeks prior to the experiment. Before use, I standardized hunger level by removing the spiders from the feeding schedule for a one-week period. Laboratory arenas were circular plastic containers containing 2 cm moist soil on the floor of the arena and 3 cm layer of thatch on the surface (as in Chapter 1). Arenas were designed to hold the search area for each predator constant for all abundance levels (Appendix: Table S2), while manipulating the number of prey in each arena. To initiate trials, crickets were released into the arenas and allowed to disperse within the arena for 15 minutes before introduction of the spiders. Individual spiders were carefully released into arenas in different locations to minimize initial interactions. All containers were placed back in the environmental chamber for a 24-hr period. Spiders were then removed, and the number of crickets remaining counted. No spiders were reused for any experiment and containers or enclosures were cleaned before reuse.

Statistical analyses

I tested for the effects of prey and predator abundance on proportion of spiders leaving the field enclosures using a generalized linear model, and consumption using a linear mixed effects model. To assess any treatment differences in proportion of spiders leaving enclosures, I implemented "glm {stats}" and specified family as quasibinomial in R (Hastie and Pregibon

1992), I used quasibinomial because of overdispersion (McCullagh and Nelder 1989). To examine differences in consumption, as measured by change in abdomen width (difference in before – after; Rypstra *et al.* 2007, and references therein), I used a linear mixed model. Specifically, I implemented the "lme {nlme}" function in R, and restricted maximum likelihood to estimate variance components (Pinheiro and Bates 2000). This allowed for partitioning the variability of the fixed effects (prey availability and predator abundance treatments) and the random effect (enclosure). To determine if cannibalism differed between the treatment groups, I used a chi-square test of homogeneity, which allowed me to test whether different treatment groups had the same ratio of spiders missing as compared to, recovered (e.g. within enclosures or in the attached cups) after the 24-hr period.

I fit three competing functional response models (Beddington-DeAngelis, Crowley-Martin, Hassell-Varley model) to the laboratory data to estimate the magnitude of interference related to predator abundance (Appendix). I did not assume *a priori* that one functional form or another would be more appropriate for the given data because the appropriate form vary between systems (Skalski and Gilliam 2001; Appendix). I fit competing models through numerical integration using "deSolve-package{deSolve}" function in R; Soetaert et al. 2010) to solve for N(t), the final prey abundance after predators were allowed to forage, while simultaneously accounting for prey depletion (Skalski and Gilliam 2001). I estimated parameters, a (encounter *rate*), *b* (handling time), and *c*, or *m* (the interference parameter), in the competing models, by maximum likelihood using the "mle2{bbmle}" function in R; Bolker 2010) with ordinary sums of squares from the numerically integrated levels of prey depletion (Skalski and Gilliam 2001). To determine the appropriate predator-dependent functional form, I compared the fit of each model using Akaike Information Criteria (AIC). Confidence intervals were determined by asymptotic estimates of standard errors using the "bbmle" package in R. I displayed the best fitting model using the per capita feeding rate (number of prey killed per predator per day) by dividing the overall feeding rate by the number of predators in a treatment. All analyses were conducted using R version 2.12.1 (R Development Core Team 2009).

Results

Interference and patch leaving

Prey availability and abundance of the predators, Pardosa milvina, significantly affected the proportion of *P. milvina* leaving enclosures (Table 1, Fig. 1a). Prey availability had the most pronounced effect on patch leaving; when prey were removed from enclosures, the proportion of spiders leaving was nearly double what was observed when prev were added to enclosures (Fig. 1a). However, spiders emigrated at a higher rate from the high predator abundance treatment when prey were added to the enclosures (Table 1, Fig. 1a). Over the 24-hr period, spiders in prey addition treatments consumed more prey, evident in the increase in abdomen width (approximately 14% increase in width), which was consistent across spider abundance treatments (Table 2, Fig. 1b). Size differences or body condition between treatments can be ruled out as a potential confounding explanation for the leaving rates and consumption because the mean size of the spiders (ANOVA carapace width: $F_{3,28} = 0.47$, P = 0.71) and condition (ANCOVA abdomen width: $F_{3,28} = 0.72$, P = 0.55) did not differ before the experiment (Appendix: Table S1). There were low levels of cannibalism as indicated by few unaccounted for spiders at the end of the 24-hr period as compared to recovered (Fig. S1). Since the differences between observed frequencies and those expected by homogeneity of responses were small, the probability of spiders missing at the end of the 24-hr period was similar between treatments ($X^2 =$ 4.59, df = 3, P = 0.20).

Interference and feeding rate

Competing functional response models consistently contained a non-zero estimate of predator interference (Table 3). This indicates that modeling *P. milvina*'s functional response was significantly improved by adding the extra parameter to account for interactions between these predators when foraging. Although all of the models converged on non-zero estimates of predator interference, the Hassell-Varely model provided the best fit to these data (Table 3). For all predator abundances, the integrated feeding rate (number of prey per predator per day) increased at low prey levels and then leveled off at an asymptote (Fig 2). The level of the asymptote decreased as spider number increased (Fig 2). The interference parameter *m* estimated was significantly greater than 1.0, which provides evidence of strong interference in this laboratory functional response experiment (Table 3). At low predator abundance, the

predators were saturated with prey and were limited by prey handling time. Increasing predator abundance, resulted in similar or lower asymptotic feeding rates as compared to the lowest predator abundance treatment (Fig. 2). The mean size of the spiders (ANOVA carapace width: $F_{11, 71} = 0.68$, P = 0.75) and condition (ANCOVA abdomen width: $F_{3, 71} = 1.01$, P = 0.45) did not differ before the experiment. Cannibalism was very low in the laboratory experiment as there were only five events (one spider missing after 24-hrs) that occurred over all the trials, (two in the four spider 40 cricket treatment, two in the eight spider 40 cricket treatments, and one in the eight spider 80 cricket treatment).

Discussion

This study provides evidence that prey abundance and interference between conspecifics are important drivers affecting foraging behavior of *Pardosa milvina*. Spiders have been thought of as self-limiting because of agonistic interactions and cannibalism (Riechert and Lockley 1984). Therefore, it would be advantageous to respond to increasing density of conspecifics by modifying space use to mediate such negative interactions. The current study provides support for this hypothesis by documenting increased patch leaving in response to conspecific abundance. In addition, the magnitude of interference was slightly higher than but similar to that predicted by ratio-dependence (where m=1), which illustrates that feeding rate is driven by a combination of prey and predator abundance. Taken together, the signal to leave patches was potentially driven by interference occurring between conspecifics that lowered foraging rate. These results provide consistent evidence of a predator dependent feedback, and emphasize the importance of understanding space use patterns in relation to functional-response characteristics of generalist predators.

Predator dependence as a process influencing functional responses is gaining recognition as an important attribute in many systems (Skalski and Gilliam 2001). However, the most appropriate form of the function response may be system specific, even though it is often assumed that the most appropriate form is the Holling Type II with interference (Skalski and Gilliam 2001). The form of functional response that best fit the feeding rate of *Pardosa milvina* on cricket prey was the modified Hassell-Varley Model (Hassell and Varely 1969; Sutherland 1983). This result indicates that when *Pardosa* are aggregated in an area foraging on prey, interference lowers foraging rate. There are multiple mechanisms that can potentially produce

predator dependence (e.g. prey refuge, wasted time, Abrams and Ginzburg 2000). The finding that *m*, the interference parameter, approximately equals one indicates ratio-dependence, where the ratio of prey to predators determines feeding rate. It is also possible that increased predator abundance induces higher prey vigilance (Abrams and Ginzburg 2000). This would lower the attack rate on prey that are hiding or less vulnerable, making it more difficult for predators attack prey. In the laboratory, I provided habitat structure that allows for high prey capture, but reduces cannibalism (Schmidt and Rypstra 2010, Chapter 1). Thus, prey would be unable to hide in this habitat. Therefore, at least for my laboratory estimate of interference, prey refuge does not seem to be the appropriate mechanism. Vagile predators such as *Pardosa* that use a sit and move foraging strategy (Samu *et al.* 2003), likely explore the available habitat in search of high harvest rates of prey. Thus, foraging is likely a dynamic process due to their high activity levels, and therefore, increased conspecific abundance appears to disrupt the foraging process to lower attack rates on prey.

Risk of cannibalism potentially influences rates of foraging and site selection. While the frequency of cannibalism was low in both the field and the laboratory experiment, cannibalism represents a risk to foraging in areas with elevated abundance of conspecifics. In the field experiment, although cannibalism was slightly higher in the high predator abundance treatment, cannibalism was similar across treatments (Fig. S1). Furthermore, because of the low levels of cannibalism measured in this study and in a prior study of *Pardosa milvina* (Buddle *et al.* 2003; Schmidt and Rypstra 2010, Chapter 1), cannibalism, at least in adults, does not appear to strongly regulate these spider populations. However, in terms of space use, even a slight amount of risk adds to the list of reasons for altering foraging tactic in response to abundance. In addition, rates of cannibalism in the genus *Pardosa* do appear to be driven by size differences between spiders (Samu *et al.* 1999). Although I successfully controlled for size (Table S3), one potential mechanistic interpretation of these data is that time lost in aggressive interactions or time lost assessing size of a potential opponent would reduce feeding rate.

Although I used a caged laboratory approach to estimate the magnitude of interference between these predators, I did not overly distort predator abundance (Abrams and Ginzburg 2000). *Pardosa* are at 100 spiders m⁻² when trophic resources and habitat structure are provided (Marshall *et al.* 2002). Furthermore, spiders in the genus *Pardosa* occur at much higher densities (600 spiders m⁻²) in other systems (Finke and Denno 2002). In addition, because I provided

preferred habitat structure (Chapter 1), this makes the experimental conditions more realistic in light of being conducted in small laboratory containers. In the laboratory I controlled for the amount of area that each individual was exposed to, and in the field both treatments had the same area, but the area per individual was reduced by increasing abundance. Although this complicates the interpretation of the effect of area on levels of interference, what is evident is that abundance levels influence foraging rate and the tendency to move to another area.

Although interference influenced foraging rate, an alternative explanation for the patch leaving response to conspecific abundance, is these spiders use one or more sensory modalities (Persons and Uetz 1997; Uetz and Roberts 2002) to assess foraging sites. In particular, *Pardosa* senses risk from intraguild predators through feces and silk produced by *Hogna hello*, which reverses their preference for complex habitat structures (Rypstra *et al.* 2007). Responses to these intraguild predators are highly tuned as they can detect predation on conspecifics in the feces of this predator (Persons *et al.* 2001). Furthermore, chemical cues from females elicits differential courtship activity and aggression (Rypstra *et al.* 2009). Other spiders use multiple sensory modalities to during mating (Gordon and Uetz 2011) and to select prey (Hostettler and Nentwig 2006). It is likely that *Pardosa* assesses the concentration of conspecific chemical signatures at foraging sites, which signals site quality. In my field experiment, the signature of multiple individuals would be very strong in the high density treatment as compared to the lower density. Furthermore, the chemical cues of prey would be very low or nearly non-existent in the prey removal treatment, and *Pardosa* would therefore perceive this area as lacking prey, leading to their decision to move to find a location containing prey or high concentrations of prey cues.

The ability of this predator to adjust its foraging in response to environmental conditions (i.e. encounter rates, time lost, risk, chemicals) is evidence of a flexible foraging.. Flexible foraging by predators is predicted to influence the dynamics of food webs (Abrams 2010; Beckerman *et al.* 2010). Resource availability as compared to interference potentially exerts effects on predators in different ways. The effects of availability may be more directly assessed by predators as the amount of energy or nutrients available, and risk and/or interference are indirect effects that reduce time spent securing resources (Schoener 1971; Mangel and Clark 1988; Preisser *et al.* 2005). My study suggests that resource abundance has a strong effect on how long these predators exert top-down control on prey, and interference competition weakens the link between prey and predators. At a local scale, increased predator abundance will result in

inference, but in open systems where predators' foraging behavior is less restricted, these predators likely attenuate interference by shifts in foraging behavior from feeding in a patch to searching for a new patch.

Combined these results provide empirical examples of an arthropod predator that uses multiple cues for determining a foraging site, and the ratio of prey to predators sets and upper threshold where these predators discontinue foraging and pursue a search strategy to find better payoffs. Understanding the interplay between predators and prey requires detailed studies that characterize numerically dominant predator species (Moreno *et al.* 2010). On a broad scale, my results suggest that emigration out of agricultural areas by common active ground dwelling predators is positively associated with increased conspecific density and reduction in prey availability. This study also highlights the complexity of generalist predator communities when behavior is included in the equation, and demonstrates the continued need to understand the factors that alter the behavior of other natural enemies.

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Table 1. Results from fitting a generalized linear model to the binomial response variable, proportion of *Pardosa milvina* leaving field based enclosures. Coefficients represent the intercept and predictor variables in the model. The prey availability treatments were either prey removed or added and predator abundance treatments were low and high abundance (7 and 28 spiders respectively).

Coefficients	Estimate	Std. Error	<i>t</i> value	Р	
Intercept	-0.99	0.16	-6.34	< 0.0001	***
Prey availability	0.90	0.21	4.15	< 0.0001	***
Predator abundance	-0.93	0.43	-2.15	0.031	*
Interaction	1.02	0.55	1.88	0.061	

Table 2. Analysis of fixed effects from a linear mixed model analysis of consumption of prey, change in abdomen width (mm), by *Pardosa milvina* in response to prey availability and predator abundance treatments in field enclosures (n = 8 per treatment combination).

	df	<i>F</i> -value	Р
Intercept	1, 149	9.51	0.002
Prey treatment	1, 23	13.22	0.001
Predator abundance	1, 23	0.01	0.926
Interaction	1, 23	1.45	0.241
Table 3. Comparison of competing functional response models used to estimate interference of the predators, *Pardosa milvina*. Values for parameters equal parameter estimates (\pm 95% confidence limits), and all were significantly different from zero, *P*<0.0001. AIC represents the Akaiki's Information Criteria and Δ AIC is displayed to compare competing models to the model containing the lowest AIC.

Model	Encounter rate (<i>a</i>)	Handling time (<i>b</i>)	Interference parameter (<i>m</i> or <i>c</i>)	AIC	ΔΑΙϹ
Hassell-Varley	0.085 ± 0.003	0.055 ± 0.002	1.175 ± 0.041	4796.93	0
Beddington-DeAngelis	0.091 ± 0.005	0.059 ± 0.003	1.515 ± 0.163	4802.67	5.74
Crowley-Martin	0.044 ± 0.001	0.023 ± 0.001	0.207 ± 0.008	5989.93	1193.00
Holling Type II	0.024 ± 0.001	0.016 ± 0.001		12176.01	7379.08

Figure 1. Patch leaving rate of *Pardosa milvina*, proportion of spiders, exiting 0.75 m² field enclosures over a 24 hr period (a), and consumption of prey measured as mean change (\pm 1SE) in the distensible abdomen width (b). For both (a) and (b) bars represent mean responses (\pm 1SE error bars) to spider abundance treatments (low (hatched bars)=7, or high (open)=28). The prey treatments on the abscissa refer to whether prey were added to enclosures or removed from enclosures prior to introduction of the spiders.



Figure 2. Results from the functional response experiment. Observed and predicted integrated feeding rates of *Pardosa milvina* at four predator abundance treatments: (a) one spider, (b) two spiders, (c) 4 spiders, and (d) eight spiders plotted against prey abundance. Open circles represent the observed feeding rates and lines represent predicted feeding rates (no. crickets captured-predator-day) from the best fitting model (see Table 2).



Appendix

Description of competing forms of functional response models (Skalski and Gilliam 2001):

All equations below represent the different functional forms, where f(N, P) represents the feeding rate. Because I did not replenish prey during experiment, prey are depleted during functional response experiments. Each functional form was fit using numerical integration over the duration of the experiment to solve for the integrated per capita feeding rate (Skalski and Gilliam 2001). In the equations below, N is the initial prey abundance treatment, P is the predator abundance treatment, f(N, P) is the instantaneous feeding rate on prey, or total number of prey killed over the one day. The parameter, a, is the attack efficiency, b, is the handling time, and c or m represents the magnitude of predator interference.

Beddington-DeAngelis (1975) model:
$$f(N,P) = \frac{aN}{1+bN+c(P-1)}$$
(1)

The Crowley-Martin (1989) model equation (2) is parameterized similarly to the Beddington-DeAngelis (1975) model equation (1) where interference, c, influences feeding rate, but predator interference is predicted to have minimal effect on feeding rate at high prey densities. Both the Beddington-DeAngelis and the Crowley-Martin reduce to Holling Type II when c = 0.

Crowley-Martin (1989) model:
$$f(N,P) = \frac{aN}{(1+bN)(1+c(P-1))}$$
 (2)

. .

In the Hassell-Varley (1990) model equation (3), the parameters *a*, *b*, and *m* are equivalent to *a*, *b*, and *c*, in the BD and CM models, respectively. The interference constant *m* indicates the magnitude of interference between predators, when m = 0, the model likewise, reduces to the traditional Holling Type II (1959). This model also allows for testing for the special case where *m* contains one in the confidence interval, or the ratio-dependent functional response (Arditi-Akacakaya 1990).

Hassell-Varley model (1990):
$$f(N,P) = \frac{aN}{bN + P^m}$$
(3)

Spider abundance treatment	Diameter of container (cm)	Area to spider ratio (cm ² /spider)
1	9.5	70.84
2	14.0	76.93
4	20.5	82.47
8	25.5	63.81

Table S1. Container size used for different densities to approximately hold the search surface area each spider, *Pardosa milvina*, was exposed to constant.

Table S2. Total number of prey added organized by prey order. Seven $1.5m^2$ samples were suction sampled from the grassy border surrounding the soybean fields located at the Ecology Research Center, Butler County. This addition of prey to mesocosms was approximately an increase in density of prey by 2316 m⁻². Standard deviation was calculated on the mean number of insects of the seven samples.

Prey order	Number of prey added	Std. dev.
Coleoptera	11	1.17
Collembola	1235	167.62
Diptera	135	7.87
Hemiptera	19	2.04
Homoptera	335	18.56
Orthopera	2	0.52

Table S3. Body condition measurements for wolf spiders in the field experiment. Body condition was measured from seven wolf spiders, *Pardosa milvina*, from each treatment. Prey treatments indicate whether prey were removed or added prior to introduction of the spider abundance treatments. Spider abundance represent low = 7 spiders or high = 28 spiders. Spiders from the high-density treatment were randomly selected from the 28 spiders initially entered into the enclosures.

Prey treatment	Predator abundance treatment	Number of spiders measured	Carapace width mean (±1std) mm	Abdomen width mean (±1std) mm
Removed	Low	56	2.13 (0.22)	2.10 (0.39)
Removed	High	56	2.14 (0.17)	2.07 (0.41)
Added	Low	56	2.09 (0.23)	1.97 (0.38)
Added	High	56	2.11 (0.19)	2.03 (0.46)

Figure S1. Mean proportion ± 1 SE of spiders, *Pardosa milvina*, cannibalized (e.g. ratio of the number missing to recovered from within the enclosures or attached cups) at the end of the 24-hr experimental period as an estimate of the frequency of cannibalism. Enclosures were the experimental unit where both prey and spiders were introduced that contained holes drilled to allow for spiders to exit into attached pitfall cups to measure emigration.



CHAPTER 3

Prey abundance effects on predation determined by ELISA-based gut content analysis of a carnivorous arthropod

Abstract

Despite the assumption that predators labeled generalist consume any prey available to them, there is a growing body of evidence suggesting otherwise. Generalists are expected to perform well in disturbed areas because they can switch between different prev pathways when one prev source is depleted. These predators have the potential to promote diversity by switching to prey in a frequency dependent manner to consume prev groups in relation to local abundance. Therefore, it is important to understand how predation fluctuates with abundance of prey in natural settings. I performed a six-month field survey and a mesocosm study in a southwestern Ohio agroecosystem to test for the role of prey abundance in determining predation frequency of a wolf spider. To quantify predation I used molecular gut content analysis, enzyme-linked immunosorbant assay with a Diptera specific monoclonal antibody. Monthly monitoring revealed that predation varied across months and the predation on flies was lower than expected when flies were in the highest abundance and higher than expected when flies were low in abundance relative to the rest of the prev community. Isolating the predators in mesocosms, showed that relative abundance influenced predation. However, spiders tested positive for Diptera at a similar frequency when flies were super abundant as compared to when flies were nearly equal in abundance relative to other prey. These spiders do not appear to be consuming flies in a strictly frequency dependent manner where the decision to switch between different prey pathways is driven by relative abundance. That Pardosa milvina selected flies independent of variation of other prey groups indicates consistent reliance on flies that may be related to some requirement of this prey source.

Introduction

Ecologists continue to be challenged to understand how resource availability influences predator-prey interactions. Historically it has been assumed that the broad diets of generalist predators is one explanation for their success in many systems because of reduced reliance on any one prey group to satisfy nutritional demands (Murdoch and Oaten 1975; Hassell and May 1986; Reichert 1999). A generalist feeding strategy is advantageous because the ability to switch between different prey types may reduce the likelihood of starvation, competition, and, at times, increase fecundity (Reichert 1984; Toft 1999; Mayntz *et al.* 2005; Hawlena and Perez-Mellado 2009). Indeed, switching between different feeding pathways is predicted to have positive effects on food web stability and community structure when predators consume the most abundant prey (Murdoch 1975; Holt 1977; Loeuille *et al.* 2010). For example, this frequency dependent prey use (reviewed by Sherratt and Harvey 1993) by generalist predators may promote more effective pest control in managed systems if their populations are sustained by a variety of alternative prey when herbivore abundances are low (Wagner and Wise 1996; Eubanks and Denno 1999; Harwood *et al.* 2004).

One of the challenges of studying predation in arthropod communities is the difficulty associated with quantifying prey capture and consumption in natural settings (Sunderland 1988; Nyffeler and Benz 1988). For one, the abundance of various prey groups varies across the season and with environmental conditions (Baggen and Gurr 1998), and it is generally assumed that changes in availability have strong effects on predation rates (Symondson *et al.* 2006). Second, observational data are difficult to obtain, especially for arthropod predators because researchers may spend thousands of hrs in the field watching for predators to catch prey to find very few predators with prey in their mouths (Nyffeler and Benz 1988; Chen *et al.* 2000; Symondson 2002). Furthermore, damage imposed on prey during mastication and digestion render any prey material observed unidentifiable (Nyffeler and Benz 1988). Molecular ecologists have developed a suite of efficient indirect post-mortem gut content analyses to reveal predation frequency on target prey by predators (Hagler and Naranjo 1994; Greenstone 1996; Chen *et al.* 2000; Symondson 2002; Harwood *et al.* 2004; Jarman *et al.* 2005; Harwood *et al.* 2007; Carreon-Martinez and Heath 2010). The benefit of these techniques is the ability to screen large numbers of field collected individuals for the presence of prey, which facilitates tracking

predation *in situ* without introducing observer interference (Symondson 2002; Harwood *et al.* 2004).

Spiders are among the most common generalist arthropod predators in terrestrial habitats (Wise 1993; Nyffeler and Sunderland 2003; Finke and Denno 2004), and considerable research has been conducted to explore their role in food webs (Finke and Denno 2005) as well as their ability to control herbivore populations (see reviews Schmitz *et al.* 2000; Halaj & Wise 2001). Yet because many species are cryptic, use extraoral digestion and masticate prey remains as they are eating, it is particularly difficult to determine precisely what they consume (Turnbull 1960; Riechert and Harp 1986; Foelix 1996; Riechert *et al.* 1999). Thus, researchers often infer trophic linkages from correlations between spider abundance and prey density (e.g. Cronin *et al.* 2004; Birkhofer *et al.* 2009). However, recent work is revealing that molecular tools can be extremely useful to examine spider diets, and feeding pathways in complex agricultural food webs (Symondson 2002; Sheppard *et al.* 2005)

The goal of this study was to examine the trophic link between a small, but common, spider and the abundance of a particular group of potential prey using a monoclonal antibody to detect prey material in the guts of these spiders. I hypothesized that the spider was an indiscriminant feeder and thus, there would be a correlation between the density of the prey group and the frequency with which it was detected in the guts of spiders in the field. I use two approaches to test this hypothesis: (1) I conducted a six month field survey of spiders and potential prey in two habitats, and (2) I manipulated the relative abundance of the target prey group in field mesocosms and allowed the spiders to forage on them for varying amounts of time. In both cases I collected the kind of prey data that are typically used to quantify spider diets and molecular gut analysis to definitively determine the frequency with which the spiders consumed my target prey.

Because they do not use webs, it is difficult to determine the diet of wolf spiders (Lycosidae) (Turnbull 1973; Uetz 1977; Foelix 1996). Yet there is continued interest in the foraging of wolf spiders, particularly those in the genus *Pardosa*, because they are often found in highly disturbed agricultural fields where they could be important for biological control (Young and Edwards 1990; Marshall and Rypstra 1999; Kiss and Samu 2000; Vogel 2004), and display an array of foraging behaviors in response to prey, habitat and competitors (Samu and Biro 1993; Samu *et al.* 2003; Chapter 1). Interestingly, the few attempts to quantify the prey *Pardosa*

capture in the field demonstrate that Diptera, predominantly free-living flies that are not agricultural pests, are an important component of their diet (Nyffeler and Benz 1988; Hayes and Lockley 1990; Bardwell and Averill 1997). Furthermore, studies have inferred a strong trophic connection between Diptera and spiders in general (Oraze and Grigarick 1989; Nyffeler *et al.* 1994; Wise *et al.* 2006); however, few studies have attempted to study this linkage using molecular tools (Harwood *et al.* 2007). Here I use a Diptera specific antibody (*DrosW-VI-B8*) to study the frequency with which the wolf spider *Pardosa milvina* consumes Diptera in Ohio agroecosystems.

Materials and Methods

Study system

Pardosa milvina is a small epigeal wolf spider that is one of the numerically dominant generalist predators in the agricultural areas of Southwestern Ohio and occurs at highly variable densities 5-100 m⁻² (Marshall *et al.* 2002). Many observational studies show that spiders in the genus *Pardosa* consume Diptera, which is a highly variable component of their diet ranging from 13-42% (Nyffeler and Benz 1988; Hayes and Lockley 1990; Bardwell and Averill 1997). These observational studies did not document prey abundance during sampling, and it is likely that some of the variability in predation is linked to changes in abundance of the prey community.

Detection limits of Diptera remains within the guts of Pardosa milvina

Laboratory feeding trials were conducted in order to determine the detection limits of Diptera proteins in the guts of *P. milvina*. Determining detection limits is important for validating the use of a molecular probe for studying predation in the field (Symondson and Liddell 1996). If the prey material does not decay following feeding, this is an indication of cross-reactivity making it difficult to determine if predators are testing positive for prey material or reacting to the probe (Symondson and Liddell 1996). I followed standard protocols for conducting feeding trials in the laboratory (Harwood *et al.* 2001; Harwood *et al.* 2004; Harwood *et al.* 2007). Specifically, I collected *P. milvina* from the Ecology Research Center (ERC), Butler County, OH between April and June 2007. To clear their guts of prey remains, spiders were housed individually in the laboratory, and provided a diet of two crickets (*Acheta domesticus*) twice per week for one month. Spiders were held in an environmental chamber set at 25 °C, 60% RH, 13:11 L: D cycle. Prior to testing, I withheld food for 1 wk. I froze 20 spiders (10 male, 10 female) that were used as controls containing no Diptera antigens. The remaining spiders were provided one *Drosophila melanogaster* (Diptera: Drosophilidae) and, if they captured it, they were allowed to feed for a 2 hr period. Feeding behavior was checked, and any non-feeding spider was rejected from the experiment. All spiders that fed were transferred to clean petri dishes. At each of five time intervals after feeding: 2, 4, 8, 12, and 24 hrs, I froze batches of 20 spiders (10 males, 10 female). Frozen spiders were stored in 1.5 ml Eppendorf tubes at -20 °C. The frozen spiders were later homogenized and screened by indirect ELISA using standard protocols (see Appendix).

Field census: prey abundance and Diptera predation

Twelve 60 m x 70 m no-till fields at the Miami University Ecology Research Center, Butler County, OH were planted on 18 May 2007. Six fields were sown with rows of soybeans (*Glycine max*, Fabaceae), and six with corn (*Zea mays*, Poaceae; see Appendix for seed and planting details).

Spider sampling. – Pardosa milvina were hand-collected from one soybean and corn field weekly between May and October of 2007. Fields were not sampled more than once in the same month. Collecting took place during the day between 1-3 pm because spiders in the genus *Pardosa milvina* are known to be diurnal (Marshall *et al.* 2002; Buddle *et al.* 2003). Prior to collection, I randomly selected a field and locality within the field by choosing random coordinates to begin searching. Sampling locations were selected as numbers between 10 m and 50 m for the width of each field and 10 m and 60 m for the length of the field. This means that anywhere that I sampled was at least 10 m from the edge of the field margin. After finding these locations, I walked through the field collecting all *Pardosa milvina* anywhere within 10 m of the edge of the field margin. I captured at least 30 individual spiders in separate 5 ml vials, and held on ice and always frozen at -20°C within 2 hrs after collection. Spiders were identified under a dissecting microscope as sub-adult or adult male and female. The mass of each individual was measured on a balance to the nearest 0.0001g, which was used to calculate the amount of PBS used to homogenize samples prior to ELISA (see Appendix). Spiders that were too young to distinguish as *Pardosa*, were discarded. Spiders were held at -20 °C until the Diptera assay (see

Appendix) was conducted to assess the predation on Diptera.

Prey abundance sampling. - Each week between May and October of 2007, I also sampled the arthropod prey community in the same corn and soybean fields to estimate abundance of prey available to P. milvina. Arthropod prey were sampled using a D-Vac suction sampler (D-Vac Company, Ventura, CA, USA). Each sample consisted of five placements of the D-Vac head for a duration of 20 second per sample (0.08 m² diameter sampling head). Random placements were selected using the same coordinate approach that I described for the spiders (i.e. five random placements were selected per field per week). Prey samples were transferred to plastic bags, and held on ice until they could be frozen at -20 °C to preserve them. I sorted and counted the prev types in the following arthropod groups: Collembola, Coleoptera, Diptera, Homoptera, Hemiptera, Lepidoptera, Thysanoptera, and Araneae. I chose these orders because they are the prey groups observed to be consumed by spiders in the genus *Pardosa* in previous studies (Nyffeler and Benz 1988). Because I was interested in how changes in Diptera abundance and the effect of relative abundance on predation frequency, for use in analyses, prev counts were converted to density (no. m⁻²), and I created three data sets: mean density of Diptera, mean relative density of Diptera (mean density Diptera/ total density of all potential prey sampled), and total prey density.

Mesocosm experiment: relative prey abundance and predation

The goal of this experiment was to use the Diptera assay to explore frequency dependent prey selection in *Pardosa milvina*. In this experiment, I looked at prey treatments defined by the combination of three relative abundance treatments of Diptera over three durations of exposure to prey treatments. Relative abundance of Diptera was manipulated using an additive design (Jolliffe 2000), where all prey treatments contained a total of 80 prey individuals representing three different ratios of Diptera to other prey. The three prey treatments were: 1) low Diptera abundance, 10 Diptera with 35 crickets and 35 leafhoppers (ratio 10:70), 2) medium Diptera abundance, 30 Diptera to 25 crickets and 25 leafhoppers (ratio 30:50), and 3) high Diptera abundance, 60 Diptera to 10 crickets and 10 leafhoppers (ratio 60:20). The time periods were 6, 30, or 72 hrs after release of spiders. To estimate prey loss during the experiment when no

spiders were present, I conducted control treatments that contained the initial medium Diptera treatment across the three time periods.

Animals used in this experiment were a combination of field collected and laboratory raised. The predators, *Pardosa milvina*, were hand-collected from fields at the ERC and held individually under standard laboratory conditions for a minimum of one month. During this time they were fed two crickets twice a week to ensure their guts were cleared of any Diptera and their hunger levels were similar. Food was withheld for one week prior to the experiment. The prey community consisted of field caught leafhoppers (*Agalia sp*, Cicadellidae: Homoptera), field caught and laboratory raised fruit flies (*Drosophila immigrans*, Drosophilidae: Diptera), and laboratory raised crickets (*Acheta domesticus*, Gryllidae: Orthopera). Leafhoppers were captured in the field using suction sampling. Flies were captured in the field and then raised on standard *Drosophila* medium (Chapter 4), and crickets were purchased from a local supplier.

I conducted this study within mesocosms, blue plastic buckets (0.067m³, diameter of 53cm, sides of 35cm in height), at the ERC between June and August 2009. Mesocosms were fitted with mesh netting (97 holes cm⁻²; No-See-Um netting, Barre Army Navy Store, Barre, VT) held in place with elastic, which prevented escape of insects and spiders. Each mesocosm was prepared with a 15 cm layer of soil covering the bottom. Four 30 day old soybean plants were transplanted from the soybean fields at the ERC into each mesocosm and a 3cm layer of wheat straw was added to cover the soil surface and provide habitat structure (Chapter 1). At the commencement of the experiment, prey were released into the mesocosms, and allowed to disperse within the mesocosm for 30min. At which point, eight spiders were released individually from plastic vials into each mesocosm. After allowing 6, 30 or 72 hrs for spiders and prey to interact, the prey and spiders were recaptured using a suction sampler. I inserted a Poulan PRO® 25cc Gas Blower/Vac with a 12cm diameter head (9335 Harris Corners Parkway, Charlotte, NC 28269), and suction sampled the prey into an insect net attached to the mouth of the vacuum head with binder clips. Suction sampling provides a reliable method for sampling predators for molecular analysis (Chapman et al. 2010). I followed suction sampling with a visual survey of any cracks and the sides to make sure that no prey or spiders remained. The entire contents of the insect net, representing one mesocosm sample, was transferred to a plastic bag and held on ice. Within two hrs of collection, the samples were frozen at -20 °C. All Pardosa milvina were sorted from the samples and placed in individual 1.5 ml microcentrifuge

tubes. Spiders were held at -20 °C until they were assessed for Diptera presence in their guts using ELISA (Appendix). Prey groups (flies, leafhoppers and crickets) were sorted and counted.

Statistical analysis

For the field survey, generalized linear models were used to explore the relationship between the binary response variable, positive tests for Diptera (measure of predation), and month sampled, habitat type (corn or soybeans), sex (male or female), age (immature or adult) of spiders, and prey abundance (covariates) using the function "glm {stats}" and specifying the family as binomial (Hastie and Pregibon 1992). To select the best variables predicting Diptera predation, I used Schwarz's Bayesian information criteria, BIC, using the "AIC" function in R and specifying k = log(n) (BIC, Burnham and Anderson 2002). Comparing BIC between models allows for selecting the simplest model that provides the best evidence of support for combinations of variables, and less evidence is present to support models when change in BIC from the model with the lowest BIC is greater than 2 (Burnham and Anderson 2002). For comparison of models, I report change in BIC (Δ BIC), and the deviance of a subset of models as compared to the null (no predictor variables), and results from fitting the best fitting model. I used ANOVA to evaluate differences in log transformed prey availability between months and habitats in the field using the function "Anova{car}" in R and reported the type III sums of squares (Fox 2008).

For the mesocosm experiment, I tested for homogeneity of prey counts across time periods by prey type (i.e. flies, leafhoppers, crickets) using a log-linear analysis. I specified a 2way contingency table (prey type and time after release) for the control treatments by fitting a log-linear model using the function "glm{stats}" in R with family set as Poisson log link (Agresti 1996). Similarly, I related the categorical variables type of prey, time after release, and prey treatments using a log-linear analysis, using a 3-way contingency table (prey type, time after release, prey treatments), and cell counts were frequency of each prey type remaining. To conduct the analysis I fit three log-linear models (full model: 3-way interaction included, reduced model: all 2-way, and additive model: no interactions) using the function "glm{stats}" in R with family set as Poisson log link (Agresti 1996; Hastie and Pregibon 1992). Using the same model selection as the field survey above, I selected the best model of these three log-linear models using Schwarz's Bayesian information criteria using the "AIC" function in R and specifying k = log(n) (BIC, Burnham and Anderson 2002).

In order to assess the effects of relative abundance of Diptera and time since release on the proportion of spiders testing positive for Diptera, I used logistic regression using the function "glm {stats}" and setting family equal to binomial (Hastie and Pregibon 1992). The response variable, positive tests for Diptera, was coded as binary, and predictor variables were: initial abundance of Diptera (as factor), time following release (as factor), and the interaction between time and prey treatments. I report the effects of initial prey abundance and time exposed to prey as odds ratios that were calculated using the function "showLogistic" in R (Hilbe 2009). The odds ratio represents a measure of how two probabilities of success differ (Collett 2003), and for my application it is the probability of testing positive for Diptera by treatment as compared to other treatments. All analyses for field and mesocosm data were conducted in R 2.12.1 (R-project.org 2010).

Results

Detection limits of Diptera remains in the guts of Pardosa milvina

The rate at which Diptera protein decayed in the guts of these spiders showed a negative exponential relationship over the time after feeding for both males and females (Fig. 1). Using the estimate of the decay constant from the regression equations, the calculated antigenic half-life (time taken to be when antigen has declined to 50% of its original concentration), was 4.91 hrs for females, and 5.30 hrs for males (Fig. 1). At 8 hrs, the probability of testing positive dropped from 100% to 75% and continued to decline to 24 hrs where the spiders were testing positive 33% of the time. These results indicate that decay of the Diptera antibody occurs rapidly in the guts of *Pardosa milvina*. Analysis of covariance indicated that there was no significant difference between males and females for which the slopes declined (natural log transformed optical density; $F_{1,10} = 0.24$, P = 0.64), but the y-intercept for males was greater than females ($F_{1,10} = 9.49$, P = 0.01; Fig. 1).

Diptera consumption in the field

Based on my survey, overall 7% of the 729 spiders sampled over the season tested positive for Diptera (Fig. 2a). Spiders of different ages or sex had similar predation frequencies

(Table S1). Prey abundance in the agricultural fields varied between months sampled (Table S2, Fig. 2b-d). Mean density of Diptera was highest in May, but there was no obvious seasonal trend (Fig. 3b). However, in relation to the rest of the prey community (relative density of flies) declined over the months sampled (Fig. 2c), and other prey increased in density from May to October (Fig. 2d).

Although I measured predation in two different field types and prey abundance varied across the months sampled, I retained only the variable month in the final model because adding any addition variables to a model containing month did not improve the fit (Table 1). This indicates that the likelihood of *Pardosa* testing positive for Diptera was not strongly linked to the prey availability measures, and spiders preyed on Diptera at similar frequencies in the two habitats (Fig. 2a). Month sampled had the strongest effect on proportion testing positive nearly seven times more frequently, and five times more frequently in September, as compared to July (Table 2, Fig. 2a).

Mesocosm experiment: relative prey abundance and predation

The number of each prey group remaining was related to the relative abundance treatments and time exposed to spiders. I was successful at maintaining approximately constant prey abundance levels over the time periods when spiders were absent from mesocosms as determined by a non-significant interaction in a log-linear analysis of homogeneity of responses $(X^2 = 0.1421, \text{ Fig. S1})$. The presence of spiders reduced the number of prey remaining across all prey types (Table S3, Fig. 3). The prey treatments, designed to manipulate the relative abundance Diptera, were maintained across time periods for all groups except Diptera, flies, as indicated by significant 2-way interactions as determined by the log-linear analysis used to compare homogeneity of responses, (prey counts) across treatments (Table S3, Fig. 3). In particular, spiders in the first 6 hrs reduced the number of flies available from 60 to 30. The number of flies remaining decreased significantly greater than predicted between 30 and 72 hrs indicated by a significant 2-way interaction between flies and 72 hrs after release (Table S2, Fig. 3). The interaction between treatment and flies indicates that counts varied by treatments greater than expected, and by 72 hrs there were very few flies remaining in any of the initial relative abundance treatments (Fig. 3). Few crickets remained at any of the time intervals, and were

lower in abundance at 30 and 72 hrs after release as compared to 6 hrs after release (Fig. 3). The decrease in numbers of crickets or leafhoppers did not differ from expected indicated by no significant 2-way interactions between these prey types and time or prey treatment (Table S2, Fig. 3). Furthermore, even though their initial abundances were equivalent, the abundance at each time period for leafhoppers was consistently higher than crickets, (t=-5.93, P<0.0001, Fig. 3). These results suggest that *P. milvina* consumed Diptera even when this prey group was very low in abundance, and were not consuming many of the leafhoppers until other prey were depleted.

Relative abundance of Diptera and time spent foraging had significant impacts on the proportion of spiders testing positive for Diptera ($\chi^2 = 16.94$, P = 0.0002, $\chi^2 = 14.47$, P = 0.0007; Fig. 4). These effects were independent as indicated by a non-significant interaction ($\chi^2 = 0.92$, P = 0.9215). Because the interaction term was non-significant, I interpreted these results as independent main effects. Specifically, increasing the relative abundance of flies resulted in an increase in predation frequency (Table 3, Fig. 4). However, increasing the abundance of flies above the medium treatment (i.e. 30:50 ratio of Diptera to other prey) did not correspond with an increase the predation (Table 3, Fig. 4). Although any spider that consumed a fly over the 6 hr period would test positive, for the other time intervals, this represents predation over approximately the last 12 hrs. Predation on Diptera declined over time, which corresponded with depletion of flies in the mesocosms. By 72 hrs, the frequency of predation was approximately 88 percent lower than at the first sampling period at 6 hrs, as indicated by an odds ratio of 0.12 (Table 3, Fig. 4).

Discussion

Foraging by this generalist predator was not totally opportunistic, but rather they exhibited sensitivity to the abundance of different prey groups and selected within the array of prey available. Here I show that it is possible to track the trophic linkage between a wandering spider and dipteran prey using a molecular analysis of predator gut contents. Over the season, the spider-Diptera consumption relationship fluctuated, but was not directly linked to changes in abundance of the prey community. Furthermore, isolating the predators in mesocosms showed that increased predation on Diptera did not tightly correspond with increase in relative abundance of this prey group. These experiments show active selection of prey, and provide a

temporal picture of predation in a natural setting. Combined these results suggest that although increased encounters with other prey should shift consumption, these spiders potentially balance intake of prey from available prey sources.

Traditionally predation is viewed as two components (Odum and Barrett 2004). The first is the rate of prey capture over time driven by prey density (i.e. the functional response, Solomon 1949). The rate of predation is expected to increase in relation to prey abundance because the encounter rate with prey increases. The second key component is the growth of the predator population in response to prey density (i.e. numerical response, Holling 1959). Combining the functional response and numerical response to prey forms a total response or recruitment of predators in response to prey (Solomon 1949; Holling 1966). To characterize the traditional views of predation requires estimating responses over given time periods related to both the rate of prey capture and population growth; however, it is often difficult to measure all of the required parameters in field experiments to examine the traditional views of foraging (Lima 2002). Estimating parameters requires accurate counts of individuals before and after some time period. Molecular analysis of gut contents provides a unique view of predation. Screening predators for prey using molecular probes opens a window to glimpse their recent foraging history. The duration the window is open, or analytical limit, is determined by the time it takes for the prey eaten to degrade in the gut of the predator (Symondson & Liddell 1993). The benefit of a molecular approach is the ability to monitor predation history of predator populations in natural settings without disrupting their foraging. In the current study, we validated the use of the Diptera antibody for predation studies in Pardosa milvina, and show that Diptera decayed rapidly within the guts of this wandering spider, which was similar between the sexes (Fig. 2), and to that measured for an unrelated group of spiders in the family Linyphiidae (Harwood et al. 2007). This short period of passage through the guts of these spiders provides reliable predation information of recent feeding activity, by limiting the possibility of detecting secondary predation (e.g. scavenging), and long decay periods may create many overlapping predation events (Harwood et al. 2001; Harwood et al. 2007; Fournier et al. 2008).

Pardosa exhibited variation in feeding frequency on Diptera over the growing season, but predation was not strongly linked to prey abundance. At the beginning of the season, Diptera composed the majority of the prey community available to this wandering spider, but by the end of the season there were few Diptera in relation to other prey (Fig. 3c). Studies have shown that

generalist predators may consume other prey when there are more alternative choices available, known as density dependent switching (Nyffeler *et al.* 1994; Abrams and Matsuda 1996; Riechert and Lawrence 1997; Harmon and Andow 2004; Harwood *et al.* 2004; Prasad and Snyder 2006). In my molecular analysis of feeding patterns this would be recognized as a significant change in positive tests for Diptera as the prey community began to shift. Although predation was variable over the season, I did not observe a shift in predation or an increase in predation related to prey availability (Fig. 3). That predation on Diptera did not change in response to abundance suggest a possible preference for Diptera in their diets, that is, although encounter rates would be low, especially later in the season relative to other prey, they did not ignore this prey group, and therefore, changes in predation are potentially driven by other prey traits or environmental conditions.

While predation was not driven by seasonal variance in prey abundance, the mesocosm study shows that *P. milvina* did increase predation on Diptera related to changes in relative abundance of Diptera, but only to a certain point (Fig. 5). At low relative abundance Diptera, spiders were potentially limited by their ability to find these prey (i.e. traditional view of predation), which would be predicted due to lower encounter rates. However, when the prey community was primarily composed of Diptera, spiders tested positive at similar proportions to when the prey community contained similar numbers of Diptera in relation to other prey groups (Fig. 5). Furthermore, that 55% tested positive for Diptera after 30 hrs in the mesocosm experiment, indicates that prey depletion was not influencing the number of predators still foraging on Diptera. Even after 72 hrs when Diptera. These results provide further evidence that prey selection in wandering spiders is independent of prey density.

That consumption of flies in an open field across the season and in a closed mesocosm setting was not tightly coupled to prey abundance, may suggest that other characteristics of prey influences prey choice in this spider. A variety of mechanisms are proposed to explain prey selection in generalist arthropod predators (Toft 1999; Eubanks and Denno 2000). Size of prey relative to predators is commonly used to gauge the strength of trophic links, which would influence both the risk of getting injured during prey capture and ability of a predator to capture larger prey (Nentwig and Wissel 1986; Brose 2010). I attempted to control for prey size by using an established sampling method that selects for small invertebrates (Brook *et al.* 2008), and

in the mesocosm study, all of the animals were similar in size. Another potential explanation is that *P. milvina* need to consume prey types to fulfill nutrient demands where at some point in the foraging process they switch to other prey to balance intake (Greenstone 1979). There is significant variation observed in nutrient composition of prey both with in and between species (Jaenike and Markow 2003; Gilbert *et al.* 2009; Wilder *et al.* 2010). Moreover, laboratory studies of spiders in this genus show that their consumption of prey is potentially strongly driven by prior experience with different nutrients within prey and the current prey available (Mayntz and Toft 2001; Mayntz and Toft 2006; Chapter 4). In my field survey of predation, I found variable consumption of flies over the season, which may relate to changes in demands for different nutrients supplied by prey sources or preference for specific species that would not be detectable using a generalized method for an entire order of insect prey.

Selection of food resources from an array of available forms is commonly deduced through counting the number consumed or remaining over a given amount of time (Chesson 1983). In field situations it is difficult to know what was consumed in relation to available. Studying prey preference in the field is further complicated by differences in the spatial distribution of predators and prey because some species of mobile predators are observed to aggregate in areas of high prey abundance (Bryan and Wratten 1984), but this is not always the case (e.g. Pearce and Zalucki 2006). Using a monoclonal antibody specific for one diverse group of prey, I found that prey abundance was not a good predictor of predation history (Table 1). Although encounter rates with our target prey group, Diptera, were decreasing over the season relative to other prey in the system, *Pardosa* continued to consume this prey group.

Spatial overlap between predators and prey is at times assumed to indicate a trophic linkage. Many papers claim that measuring prey availability using a D-Vac provides an accurate measure of prey available for mobile arthropod predators (Denno *et al.* 2003; Showler *et al.* 2003; Cronin *et al.* 2004; Koss *et al.* 2004; Brook *et al.* 2008), but here I show that the prey available, determined by this approach, did not relate to foraging patterns in a density dependent manner. Therefore, spatial overlap may not inform us of the strength of food web links. However, the benefit of using molecular techniques is that you can say what a predator selected even in situations where direct observation is difficult or if other factors influence predator aggregation more so than prey abundance (e.g. habitat complexity, see review Langellotto *et al.* 2004). The current limitations of molecular techniques are that further modeling is needed to convert predation data into magnitudes of interaction strength between species and into an understanding of population dynamics (Naranjo and Hagler 2001).

Evidence suggests that spiders in the genus *Pardosa* provide pest control as natural enemies in agricultural systems. For example, these spiders are potentially important for control of medfly pests in citrus orchards (Monzo *et al.* 2010), and silk production has indirect effects on prey that lower plant damage (Hlviko and Rypstra 2005), however more work is needed to understand their role as a natural enemy for specific pests in different agricultural settings. Studies show that *Pardosa* reduces aphid populations in the laboratory in winter wheat (Marc *et al.* 1999) and can depress numbers of leafhoppers in corn crops (Lang *et al.* 1999). Here I show that predation on Diptera in two agricultural contexts, soybean and corn fields was similar (Fig. 2). Because of the resolution of the molecular probe (i.e. order specific), I am unable to say for sure if these predators altered their foraging to a more specific taxonomic level. Diptera is known to be one of the most diverse insect taxa with species spanning the spectrum of ecological roles in insect communities (Yeates *et al.* 2007). *Pardosa milvina* may prefer one species or group of flies over others, which I was unable to detect using an order specific gut analysis.

Pardosa milvina displayed less interest in a common herbivore, *Agalia* sp. (leafhoppers), in comparison to the other prey types provided (Fig. 4), which suggests that leafhoppers are a less preferred prey source in this system. For instance, *Pardosa milvina* began to depress the abundance of leafhoppers only after 30-72 hrs when flies and crickets were nearly eliminated from the mesocosms (Fig. 4). These results reiterate a common thread present in my results, and that is, predation by species in the genus *Pardosa* is species and system specific. In some systems, *Pardosa* consume high numbers of herbivores and high levels of these predators promote plant growth (Döbel and Denno 1994; Gratton and Denno 2003; Cronin *et al.* 2004). It may be that ground dwelling predators primarily consume prey that are on the ground (Bardwell and Averill 1997) or act synergistically with predators that are up in the vegetation that dislodge prey making them available to ground foraging predators (Losey and Denno 1999). The trouble with determining the role of predation in structuring communities, is that studies commonly do not know what the predator was consuming. Therefore, unable to say for sure if consumption lowered the abundance of a given group, or whether the predator free space (Abrams 2007).

Using molecular tools in ecological contexts allows for a robust diagnosis of how predation is varying over the landscape in response to ecological conditions (e.g. Winder *et al.* 2005).

The peaks of abundance of males and females overlap over the growing season (Marshall et al. 2002), and it is possible that the drop in predation in July could be linked to the phenology of *Pardosa milvina*. Around July, reproductively active females have been shown to be low in abundance relative to early August. Lower abundance observed in Marshall et al. (2002) would be related to activity patterns because pitfall traps were used to assess abundance. Lower activity may correspond with lower levels of foraging and potentially Pardosa seeking refuge to procure spiderlings. Interestingly, my study suggests that consumption of prey by males and females is equivalent over the season (Table S1, Table 1). This is surprising because males are shown to eat less in the laboratory under variable prey densities, which provided a clear distinction in their functional responses (Walker and Rypstra 2002). Furthermore, no observed sexual differences in consumption in relation to the prey availability, in my field survey, suggests that both sexes consume similar types of prey or at least a similar amount of Diptera. Further study is needed to clarify the specific types of Diptera consumed, and test the hypothesis that males and females exhibit similar trophic linkages. Currently underway is the development of specific DNA primers to study species specific linkages in food webs (see review Sheppard and Harwood 2005; Harwood et al. 2009), which will allow for future research to disentangle these complex food webs, and examine the strength of species specific linkages.

With the wide spread populations of *Pardosa* (Nyffeler and Sutherland 2003; Denno *et al.* 2003; Vogel 2004; Monzó *et al.* 2009), further studies are needed to characterize at a finer scale the shifts in predation from one prey source to another. While it is recognized that predators may switch between prey types, more studies are needed to document changes in foraging on prey in relation to both predator and prey abundances (Cardinale *et al.* 2003; Bruno and Cardinale 2008; Szendrei *et al.* 2010). While there was not a clear connection in how prey abundance drives the consumption of Diptera, that in a given month upwards of 25% of spiders tested positive indicates that Diptera is an important food group. My study suggests that variation in abundance of multiple prey groups is not enough to explain the predation rate on at least one food web link in this system. Changes in behavior of predators or prey may alter space use (Abrams 2007), hence make it difficult to discern the predictive power of abundance to the

foraging behavior of predators because prey may be avoiding predators, while predators chase prey around in the landscape. However, to robustly test this hypothesis requires further study and development of specific molecular probes to examine more prey species using a spatially explicit approach to sampling both predators and prey.

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Table 1. The results of model selection to determine the best fitting combination of variables explaining the proportion of *Pardosa milvina* testing positive for Diptera in their guts over a growing season in soybean and corn fields at the Ecology Research Center. The Bayesian Information Criteria (BIC) was used to weigh evidence in support of competing models, where the lowest BIC indicates the best model. Change in BIC (Δ BIC) from the best fitting model, and change in deviance (Δ Deviance) from the null model that included no predictor variables, were used to compare models containing different combinations of variables.

Variables in model	BIC	ΔΒΙϹ	∆Deviance null model
Month	395.48	0	-23.89
Month + total prey density	398.28	2.8	-24.01
Month + density Diptera	398.38	2.9	-24.81
Total prey density	399.19	3.71	-2.17
Month + relative density Diptera	399.36	3.88	-23.89
Relative density Diptera	401.34	5.86	-1.44
Density Diptera	402.91	7.43	-0.15
Age + sex + habitat + month	404.84	9.36	-26.4
Age + sex + habitat	407.95	12.47	-3.16

Table 2. Results from the best fitting generalized linear model containing the predictor, month sampled, on proportion of spiders, *Pardosa milvina*, testing positive for Diptera in their guts determined by ELISA with a Diptera specific monoclonal antibody. Each month was coded as indicator variables with July as the reference. LCL and UCL represent the lower and upper confidence limits of the odds ratios, and SE represents the standard error of the estimated coefficient.

	Odds ratio	LCL	UCL	Estimate	SE	z value	Р
(Intercept)		-	-	-3.64	0.45	-8.03	< 0.0001
May	6.85	2.39	22.47	1.92	0.56	3.44	0.001
June	4.67	1.86	14.22	1.54	0.51	3.03	0.002
August	3.74	1.09	13.38	1.32	0.62	2.12	0.034
September	4.81	1.65	15.86	1.57	0.56	2.79	0.005
October	1.03	0.21	4.27	0.03	0.74	0.04	0.971

Table 3. Results from generalized linear model evaluating the effects of relative abundance of Diptera(low, medium, high), and time foraging (6, 30 or 72 hrs) in field-based mesocosms on the proportion of spiders, *Pardosa milvina*, testing positive for Diptera in their guts determined by ELISA with a Diptera specific monoclonal antibody. Prey treatments and time were coded as indicator variables with high relative density of flies and 72 hrs coded as reference levels. I indicate the comparison being made in the table for each treatment group. I report the LCL and UCL, which represent the lower and upper confidence limits of the odds ratio. The Est. with SE represents the estimate of the coefficients for each indicator variable and associated standard error of the estimate.

Comparison	Odds ratio	LCL	UCL	Est.	SE	Z	Р
Low vs. high	4.54	2.48	8.57	1.51	0.32	4.80	< 0.0001
Medium vs. high	0.96	0.55	1.69	-0.04	0.29	-0.13	0.8975
6 hrs vs. 72 hrs	0.12	0.05	0.24	-2.11	0.38	-5.59	< 0.0001
30 hrs vs. 72 hrs	0.24	0.10	0.52	-1.44	0.41	-3.47	0.0005
Figure 1. Decay curves of Diptera antigen to validate the use of the Diptera specific monoclonal antibody for use in *Pardosa milvina*. Each curve represents the relationship between time after feeding and change in the concentration of Diptera antigen, as measured by optical density with error bars representing (\pm 1SE), in the guts of female y = 0.506e^{-0.129x} (a) and male y = 0.709e^{-0.135x}, (b) *Pardosa milvina* (n = 10 per time period per sex).



Figure 2. Temporal changes in predation and prey density in two agricultural field types sampled at the Ecology Research Center. Mean monthly proportion *Pardosa milvina* testing positive for the presence of Diptera within their guts (a), mean density of Diptera (b), mean relative density Diptera (c) and mean overall prey density (d) as related to habitat and month sampled. Bars or symbols represent the mean response (± 1 SE). A total of 729 spiders were sampled and mean prey density represents 5 samples taken each week within each month.



Figure 3. Change in prey abundance at time intervals following release of female *Pardosa mivlina* into field-based mesocosms as related to prey treatments. Mean counts of each prey species are represented by different letters (a) flies, (b) leafhoppers, and (c) crickets. Symbols indicated by the legend represent initial relative abundance of flies: low relative abundance (10:70), medium (30:50), and high (60:20). Each time listed on the abscissa represents the destructive sampling point for a time interval. Symbols represent mean (± 1 SE) (n = 8/treatment combination).



Figure 4. Mean proportion ± 1 SE of eight female *Pardosa milvina* testing positive for the presence of Diptera within their guts, determined by ELISA, as a function of time following release (n = 8/ treatment combination). Symbols indicate mean proportion with bars representing ± 1 SE of spiders testing positive for flies in their guts by prey treatments: low (10:70), medium (30:50), and high relative abundance of flies to other prey available (60:20).



Appendix

Description of fields

Corn fields - Steyer Seed corn 1095 VT3 was planted in no-till fields on 5/18/07. Seeds were planted at 67,284 seeds per hectare spaced 0.61 m rows. Starter fertilizer (18-46-0) was spread at 252 kg per hectare, broadcasted potash (0-0-60) was broadcasted at 224 kg per hectare with a 0.61 m by 0.61 m placement, and 28% nitrogen was side dressed at 421 l per hectare. *Soybean fields* - Ebbert's seed 1365RR 3.6 Tall, bushy with cyst resistance was planted in no-till fields on 5/18/07. Seeds were planted at 358, 024 seeds per hectare with a 0.61 m by 0.61 m row placement. On 5/11/07 and 6/12/07, the fields were sprayed using burn down sprayer of Buccaneer PlusTM at 2.32 l per hectare to control weeds.

Description of ELISA gut content analysis

Pardosa milvina from these experiments were homogenized and screened using standard protocols for enzyme-linked-immunoglobulin assay, ELISA (Sydmonson et al. 2002, Harwood et al. 2004, Harwood et al. 2007) with a Diptera-specific monoclonal antibody, DrosW-VI-B8 antibody (Harwood et al. 2007). Spiders were homogenized in 1:20 (mg:µl) with PBS (phosphate-buffered saline pH 7.4). Each homogenized sample was then centrifuged at 8000g for 15 min, and the supernatant was transferred to a clean 1.5 ml microcentrifuge tube and stored at -20°C. The remaining solids were discarded. To screen spiders via ELISA, 200 ul of each sample of homogenized spider was coated in duplicate onto 96-well microtitration plates at a concentration of 1:20,000. Four positive controls coated on each plate and eight Non-Dipteran negative controls that were determined to elicit the highest absorbance to the DrosW-VI-B8 antibody (Harwood et al. 2007). Following overnight incubation, wells were washed three times with PBS Tween (0.05% polyoxyethylene-20 sorbitan monolaurate; Sigma–Aldrich, St. Louis, MO). One well of each sample and control was coated with the DrosW-VI-B8 antibody diluted 1:1000 in PBS Tween and the other well for each was coated with PBS Tween (which allows for subtracting any non-specific binding that occurs). Plates were then incubated for 2 hr, after which, the plates were washed again three times with PBS Tween and coated with PBS Tween and ImmunoPure® goat anti-mouse IgG horseradish peroxidase enzyme conjugate (Pierce Biotechnology, Rockford, IL, USA), diluted 1:4,000 in PBS Tween and incubated for 1 hr. Following this period, plates were again washed three times with PBS Tween and coated with the enzyme substrate, o-phenylenediamine (Sigma-Aldrich, St. Louis, MO, USA). After a 30 min incubation period in the dark, the reaction was stopped using 50 ul 2.5 H₂SO₄ and the absorbance was recorded at 492 nm using a spectrophotometer (Thermo Electron Corporation, Waltham, MA, USA). Pardosa milvina were scored positive for the presence of Diptera if their recorded absorbance was greater than the mean + 3.0 SD of the absorbance measured for the negative controls (Harwood et al. 2007).

Table S1. Summary of the overall proportion of female and male Pardosa milvina in different
age classes (penultimate, fully developed adults) testing positive for the presence of Diptera
within their guts, determined by ELISA using a Diptera-specific monoclonal antibody.

Sex	Age	Mean proportion positive	Std.Error
Female	Adult	0.09	0.02
Female	Immature	0.08	0.02
Male	Adult	0.09	0.03
Male	Immature	0.04	0.02

Source	df	F	Р
Mean density of Diptera	-		
Month	5	29.60	< 0.0001
Habitat	2	11.24	0.0036
Month*Habitat	10	36.76	< 0.0001
Residuals	240	482.36	
Relative density Diptera			
Month	5	59.84	< 0.0001
Habitat	2	1.93	0.3808
Month*Habitat	10	20.03	0.0290
Residuals	240	75.49	
Mean total density of prey			
Month	5	98.26	< 0.0001
Habitat	2	3.494	0.1743
Month*Habitat	10	28.936	0.0013
Residuals	240	149.23	

Table S2. Results from ANOVAs used to test for the effects of month and habitat on the density of Diptera, relative density of Diptera or total prey density as related to the factors habitat where samples were taken and month samples were taken from fields a the Ecology Research Center. All density response variables were natural logarithm transformed prior to the analysis.

Table S3. Model selection of best fitting log-linear model that maintained hierarchical structure (a). Models were ranked by BIC values, Schwarz's Bayesian information criteria, and less evidence is present to support models when change in BIC, Δ BIC>2. The results from the best fitting log-linear analysis (b) to test for homogeneity of prey remaining across the categorical predictors prey treatments and time exposed to spiders, *Pardosa milvina*, in field-based mesocosms. Variables in model were prey type (fly, leafhopper, cricket), time (6, 30 72 hrs), and prey treatments (low, medium, high). All were coded as categorical to form a 3-way contingency table with cell counts represented by prey remaining after each time period by treatment combination. SE represents the standard error of the estimated coefficient in a model.

Models	BIC	ΔΒΙϹ
Reduced model (all 2-way interactions)	287.39	0
Saturated model (3-way interaction): time*treatment*prey type	335.04	47.65
Additive model: time + treatment + prey type	864.46	577.07

(b)

(a)

Variables	Estimate	SE	z value	Pr(> z)
(Intercept)	3.59	0.14	25.82	< 0.0001
Flies	2.04	0.15	13.93	<0.0001
Leafhoppers	0.79	0.16	4.95	<0.0001
30 hrs	-0.63	0.15	-4.20	<0.0001
72 hrs	-1.56	0.22	-7.03	<0.0001
Low relative abundance	1.02	0.16	6.53	<0.0001
Medium relative abundance	0.74	0.16	4.63	<0.0001
Flies*Low relative abundance	-3.13	0.20	-15.47	<0.0001
Leafhoppers*Low relative abundance	-0.14	0.17	-0.79	0.427
Flies*Medium relative abundance	-1.38	0.17	-8.00	<0.0001
Leafhoppers*Medium relative abundance	0.09	0.18	0.50	0.615
Flies*30 hrs	-0.03	0.15	-0.18	0.858
Leafhoppers*30 hrs	0.19	0.13	1.38	0.168
Flies*72 hrs	-1.47	0.25	-5.79	<0.0001
Leafhoppers*72 hrs	0.22	0.17	1.30	0.194
30 hrs* Low relative abundance	0.23	0.14	1.67	0.094
72 hrs* Low relative abundance	0.35	0.21	1.65	0.098
30 hrs*Medium relative abundance	0.01	0.12	0.11	0.913
72 hrs* Medium relative abundance	0.53	0.20	2.62	0.009

Figure S1. Abundance of prey groups over time in field mesocosms. These were controls for monitoring prey abundance in mesocoms without the presence of spiders, *Pardosa milvina*, in the mesocosms. Results show, that without spiders present, prey levels remain fairly constant over time. Bars represent mean ± 1 SE (n=8 per time period).



CHAPTER 4

Nutritional content of prey affects the functional response and prey preference of a generalist arthropod predator

Abstract

Predators can have a large impact on the structure and function of communities and ecosystems. However, relatively little is known about how nutrition affects the impact of carnivores on prev populations, especially in terms of the amount of prey consumed (e.g., the functional response) and choice of prey. I manipulated the nutrient status of a single species of prey and tested the effects of current prey quality and prior feeding history on the functional response and prey preference of the wolf spider, Pardosa milvina. Proportion of prey killed was consistent with a type II functional response when exposed to either low or nutrient enhanced prey; however, spiders ingested more of the low nutrient prey. Conversely, prior feeding history shifted the functional response, and spiders previously fed prey higher in nitrogen killed a greater proportion of prey during the functional response, but the amount of prey actually consumed was similar. Feeding history also altered preference for prey. Spiders fed nutrient enhanced diets, were more selective and preferred the same nutrient enhanced prey as they experienced during the threeweek conditioning period. Spiders conditioned on a low nutrient diet, containing lower amounts of protein and other nutrients, did not exhibit preference for prey. These results highlight the importance of examining the nutritional content of prey for carnivores and better incorporating nutrition into the study of predator – prey dynamics.

Introduction

Generalist predators can have a large impact on the structure and function of communities and ecosystems (Schmitz *et al.* 2000; Schmitz *et al.* 2010). The impact of such broad-based carnivores on food webs is due both to their behavioral effect on prey habitat choice and foraging, and their quantitative effect on prey abundance (Peacor and Werner 2001; Werner and Peacor 2003; Schmitz *et al.* 2004). In some systems, the quantitative effect of predators on prey populations can be quite large, and has been modeled and studied in terms of its functional response, which is the relationship between predator consumption and prey density (Holling 1959). The functional response continues to remain relevant in studies of consumer interactions (Beckerman 2005; Bollache *et al.* 2008), and is especially important in studies predicting and modeling the impact of predators on prey populations (Englund and Leonardsson 2007; Okuyama 2009), and adaptive choices made by predators to variation in prey traits (Abrams 2007).

Functional responses have been studied in many systems for decades (Holling 1966; Dale, Adams and Bowyer 1994; Iason et al. 2002; Pitt and Ritchie 2002), though relatively few empirical studies have considered how variation in prey attributes affect the functional response (Abrams 2010). Classically, predators were thought to be limited by capture success related to prey abundance (Solomon 1949; Holling 1966; Oaten and Murdoch 1975; Wise 1993), and assumed to be indiscriminate because prey may be of similar value (Wise 1993; Toft 1999). However, adaptive foraging models predict that the diet of predators is altered by a combination of prey availability and energetic benefits of prey (Whelan and Schmidt 2007). On the other hand, there is increasing evidence that the nutritional content of prey items could also limit the growth and abundance of a wide range of animals including carnivores (White 1978; Fagan et al. 2002; Mayntz et al. 2005; Bukovinszky et al. 2008). Recently more and more studies have focused on the movement of specific elements or nutrients through food webs (Raubenheimer and Simpson 1997; Sterner and Elser 2002; Raubenheimer, Simpson and Mayntz 2009). These studies have revealed that even a relatively small variation in the nitrogen or protein content of food items can have significant impacts on the performance of animals, their diet choice and, by extension, their role in the ecosystem. For example, small shifts in the diet fed to insect prey that are then provided to spiders have been demonstrated to influence aspects of their growth, development, mating behavior, and survivorship of the spiders (Toft and Wise 1999; Mayntz and Toft 2001; Mayntz and Toft 2006; Wilder and Rypstra 2009; Lomborg and Toft 2009). Given the importance of spiders in terrestrial food webs (Chen and Wise 1999; Denno *et al.* 2003; Nyffeler and Sutherland 2003; Oelbermann and Scheu 2009), it is critical to determine if differences in the nutritional status of these predators or differences in nutritional status of potential prey shift the functional response and alter prey preferences.

Nutritional studies suggest that prey choice is important but it is difficult to determine if spiders can select prey based on differences in their nutrient content alone. Early studies of nutrient balancing in spiders conducted by Greenstone (1979) suggest that at least one spider, *Pardosa ramulosa*, potentially consumes prey from different arthropod groups to balance their consumption of amino acids. More recently Mayntz *et al.* (2005) demonstrated that spiders can alter their intake in response to their particular nutritional needs. Therefore, evidence is building that even these generalist predators exhibit discrimination of prey to potentially optimize their diets in favor or increased growth, survival, and fitness (Toft *et al.* 2006). Understanding nutritional aspects of prey choice is therefore necessary to predict the effect of carnivorous arthropods in food-webs to better understand how prioritization of available nutrients influences food-web structure (Denno and Fagan 2003).

I tested the hypothesis that prey quality affects the functional response and prey choice of the common wolf spider, *Pardosa milvina* (Araneae: Lycosidae). Previous work has demonstrated that rearing fruit flies, *Drosophila melanogaster*, on media supplemented with different nutrients affects the quality of this prey item for spiders in the genus *Pardosa* (Mayntz and Toft 2001; Mayntz *et al.* 2005; Wilder and Rypstra 2008). As a result, this system provides a convenient way to alter prey quality, while preserving other aspects of prey morphology and behavior that might influence the predator-prey interaction. I conducted three experiments: (1) first I tested if the nutrient content of prey provided to spiders (either low or nutrient enhanced fruit flies) affected the functional response or consumption of *P. milvina*, (2) then I maintained spiders on either a low or nutrient enhanced diet for a period of time prior to quantifying their functional response and consumption of a single prey type, and, finally, (3) I tested if spiders could discriminate and select between fruit flies that differed in nutritional content when different types were provided together.

Materials and methods

Study system

Wolf spiders in the genus *Pardosa* are common generalist predators in agroecosystems throughout the world (Nyffeler and Sutherland 2003; Vogel 2004; Monzó *et al.* 2009). The wolf spider, *Pardosa milvina* was selected as the predator for these experiments because it is the numerically dominant predator in agroecosystems of the Southwestern Ohio, USA (Marshall, Pavuk and Rypstra 2002), its functional response has previously been characterized as type II (Walker and Rypstra 2002), and because its growth, feeding, and mating behavior are known to respond to variation in food quality (Mayntz and Toft 2001; Mayntz and Toft 2006; Wilder and Rypstra 2009). Penultimate or adult female *Pardosa milvina* were collected from the Ecology Research Center at Miami University, Butler County, Ohio (39°31'42"N, 84° 43'48" W) between May and August 2006 for the functional response tests, and between May and August 2007 for the choice tests. Once in the laboratory, *Pardosa milvina* were housed individually in plastic containers (6 cm diameter and 4 cm sides) with a 15 cm moist soil substrate, in an environmental chamber (25°C, 50-58% RH and 13:11 L:D cycle), fed two 0.32 cm crickets, and watered twice per week until they were randomly assigned to treatments.

For all experiments I used vestigial-winged fruit flies, *Drosophila melanogaster* (Diptera: Drosophilidae), as the prey species. Studies have shown that Diptera may be an important component in the diet of wolf spiders (Nentwig 1986), and the nutritional content of Diptera can vary substantially in the field (Markow *et al.* 1999; Jaenike and Markow 2003). I used a common model system for manipulating the nutrient content of *D. melanogaster* used as prey for spiders (Mayntz and Toft 2000, 2001; Mayntz *et al.* 2003; Jespersen and Toft 2003; Mayntz and Toft 2006; Wilder and Rypstra 2008; Lomborg and Toft 2009). Individuals of *D. melanogaster* were raised on either a regular media, referred to as "regular prey" (i.e., potato flakes with an antifungal compound; Ward's™ Natural Science, Instant *Drosophila* Medium, Natural Science, Rochester, NY, U.S.A.), The second prey type was "nutrient enhanced", which consisted of the same basic medium supplemented with 40 % dogfood by mass (Ol' Roy™ Dog Food; 27% crude protein, 15% crude fat, 4% fiber, 1.2% Calcium, 0.9% Phosphorus, and other nutrients; Wal-Mart, Bentonville, AR, USA). The use of the terms "regular" and "nutrient enhanced" for the treatments is in reference to nutrients added to the media and presumably found in the bodies of the adult flies (see Table S1). This identical manipulation has been used

in a number of previous studies and, in every case, performance (e.g., growth and reproduction) of spiders fed "nutrient enhanced" flies is significantly higher than performance of spiders fed "regular" flies (Mayntz and Toft 2000; 2001; Mayntz *et al.* 2003; Jespersen and Toft 2003; Mayntz and Toft 2006; Wilder and Rypstra 2008; Lomborg and Toft 2009). In particular, adult female *Pardosa milvina* (the developmental stage and species used in the current study) produce egg sacs significantly faster when fed the nutrient enhanced compared to the regular *D. melanogaster* (Wilder and Rypstra 2008).

I compared the mass of flies raised on the regular and nutrient enhanced flies to ensure that the diet manipulations did not affect the overall mass of prey available to spiders. To compare differences between the treatments in fly size I sub-sampled five individual flies from seven culture bottles for each treatment. The individual flies were dried at 60 °C for 48 hrs and weighed to the nearest 0.001 mg on a microbalance. I tested for differences in the dry mass of flies reared on the two prey types using a one-factor analysis of variance with media as a main effect and culture as a blocking factor. To conduct the nutrient analysis of flies reared on the two media, I collected flies from 19 regular cultures and 20 nutrient enhanced cultures. Flies were collected from culture bottles that were set up over a period of several months. After drying flies at 60 °C for 48 hrs, I packaged 2 – 3 mg of flies (ca. 6 - 8 individuals from a single culture bottle) using a CHN analyzer (Perkin Elmer, Boston, MA, USA) to measure carbon and nitrogen content (see Table S1 in Appendix). Using data on the nitrogen content found in the *D. melanogaster*, I converted nitrogen content to protein using the standard conversion factor of 6.25 (Horowitz 2002; Mayntz *et al.* 2005; Wilder *et al.* 2009). I tested for differences in protein content using a one-factor analysis of variance to compare protein content between prey types.

General methods

All experiments were conducted in circular plastic arenas (20 cm diameter, 10 cm deep) with a 2 cm layer of moist potting soil mixture covering the bottom, and a 3 cm layer of artificial straw (Textraw® Synthetic Straw, St. Simons Island, GA, USA) added to provide structure. Immediately prior to commencing the experiment, I verified that spiders assigned to treatments were the same size and in similar condition by measuring the carapace and abdomen width of all *P. milvina* using a digital micrometer attached to a stereomicroscope, accurate to ± 0.01 mm. These measurements were used to assess body condition of individuals, specifically, an

ANCOVA of abdomen width, with size (carapace width) as the covariate (Rypstra *et al.* 2007). At the commencement of each trial, I first released flies, and allowed them to disperse for 15 minutes before I introduced a single spider. The container was closed, and returned to the environmental chamber. After 24 hrs, I removed the spider, re-measured the abdomen width, and counted prey alive. Since the container was closed and the flies could not fly (i.e., they were vestigial-winged), I assume that all flies missing were killed by the spider (Schmidt and Rypstra 2010). In spiders, the size of the cephalothorax is fixed but the size of the abdomen varies depending upon the amount of food consumed (Foelix 1996). Hence I used the difference in abdomen width before and after the trials as a measure of consumption over the 24 hr period (see Rypstra et al. 2007 for a detailed description). Differential digestibility of low and nutrient enhanced flies is unlikely to have biased our measurements of abdomen width as a proxy for the biomass of flies ingested (Jensen et al. 2010). A study of a congeneric spider, P. prativaga, showed that once spiders ingest nutrients and attain a particular mass, the rate at which body mass is lost over a short term period (i.e., < 5 days) is independent of the nutrient content of the prey (Jensen et al. 2010). Spiders were never reused within or between experiments, and containers were cleaned with detergent, rinsed, wiped down with alcohol, and allowed to dry completely before reuse in subsequent trials.

Functional response: prey quality and feeding history

Two functional response experiments were conducted: (1) to test if prey quality affected the functional response (number of prey killed and amount ingested) of spiders, and (2) to test if the prior diet of spiders affected their functional response when fed on a common prey type. In both experiments I characterized the functional response over the same five prey densities (10, 20, 30, 40, 50 flies; n=10-14/treatment combination). In the first experiment, spiders were fed two 0.32 cm crickets, twice per week for two weeks prior to the experiment. I measured the functional response of *P. milvina* on each prey type (regular and nutrient enhanced). In a second experiment, I altered the feeding history of spiders by placing them on diets of either two low or two nutrient enhanced flies twice per week for three weeks prior to the start of the experiment. I characterized the functional response of both feeding history groups using nutrient enhanced flies as prey.

Prey choice: Prey quality and feeding history

In order to determine if feeding history influences prey choice, I conducted choice tests in which spiders were allowed to forage on a mixture of equal proportions of low and nutrient enhanced prey. To distinguish between prey quality groups, I used two vestigial winged fruit fly mutants, red-eyed and white-eyed. To determine if *P. milvina* had a mutant preference related to quality, first a choice test was conducted using two combinations of flies (n=11/combination). One combination contained 20 white-eyed regular flies mixed with 20 nutrient enhanced red-eyed, and the second combination contained 20 white-eyed nutrient enhanced flies mixed with 20 regular red-eyed flies. Spiders fed on our standard laboratory diet of two 0.32 cm crickets, twice per week were allowed to forage in these mixed prey quality environments for a 24-hr period then I removed the spiders, and counted the flies remaining alive.

Once I established that spiders did not have a preference for specific fly eye color mutants (see results), I repeated the mixed prey choice on two contrasting diet treatments. I manipulated prior diet of spiders, in the same way as the functional response experiment, over a three-week period where spiders were fed two low (n=20) or two high (n=21) quality flies twice per week. Spiders were allowed to forage in the mixed prey quality environment containing 20 white-eyed regular flies mixed with 20 nutrient enhanced red-eyed flies. Following a 24-hr period the spiders were removed, and each fly type remaining alive was counted.

Statistical Analyses

Functional response experiments - Logistic regression was used to determine the functional response of the proportion of prey killed (N_e/N_0 ; where N_e = number killed, and N_0 = initial number of flies) to the initial number of prey (N_0) present and a higher order term (quadratic = N_0^2) were used to account for nonlinearity (Juliano 2001) using PROC LOGISTIC with a binomial error distribution. To distinguish between different functional forms I used maximum likelihood to test if coefficients (i.e. $\beta_1 N_0$ and $\beta_2 N_0^2$) were significantly different from zero, then compared the sign on each coefficient. I determined the functional response was type I if the coefficients were not significantly different from zero, type II if the linear term (N_0) was negative (decreasing), and type III if the linear term was positive (increasing). Differences between prey type or prior diet treatments in the proportion of prey killed was determined using indicator variable for prey type or prior diet (Juliano 2001; Walker and Rypstra 2002).

The functional response parameters (attack constant, *a*, and handling time, T_h , of prey) were obtained using nonlinear least squares and using Newton's method for parameter estimation (PROC NLIN, SAS 9.2, SAS Institute, Inc., Cary, IN USA) to estimate these parameters from the random predator equation (Juliano 2001). The random predator equation is:

$$N_{e} = N_{0} \Big[1 - e^{a(T_{h}N_{e} - T)} \Big]$$
 eqn 1

where N_e is the number of flies killed, N_0 is the initial fly density, *a* is the attack constant (related to encounter rate), T_h is handling time and *T* is total time. To determine if either the prey type or the prior diet treatments influenced the functional response parameters, indicator variables were added to the implicit function, which allows comparison of parameters within each experiment using a t-test and confidence intervals (for details see Juliano 2001). Functional response analyses were conducted in SAS 9.2 (SAS Institute, Inc., Cary, IN, USA).

To understand the impact of different prey quality types and prior diet on consumption of prey, I assessed change in abdomen during the 24 hr period between treatments for each of the functional response experiments. Body measurements are commonly used to monitor recent consumption of prey, and the abdomen of spiders is a flexible part and increases in size when the spider feeds (Rypstra *et al.* 2007). ANOVA was used to compare consumption, natural logarithm transformed change in abdomen, between prey abundance treatments and prey nutrient treatments, for each experiment. Consumption analyses were conducted in JMP 8 by implementing "Fit model" and specifying personality as standard least squares (SAS Institute, Inc., Cary, IN, USA).

Prey choice experiment - Selectivity for prey quality type when spiders were exposed to a mixed prey quality environment was assessed using Manly-Chesson selectivity index, α selectivity metric (Manly *et al.* 1972, Chesson 1983). This is an appropriate preference measure in situations with prey depletion and when prey remaining is ≥ 10 . Any trial that did not contain a minimum of ≥ 10 prey items at the end of the 24 hr period was discarded. The equation to estimate α is:

$$\alpha_{i} = \frac{\ln[(n_{i0} - r_{i})/n_{i0}]}{\sum_{j=1}^{m} \ln[(n_{j0} - r_{j})/n_{j0}]}$$
eqn 2
$$i = 1, m = 2$$

where n_1 and n_2 equal the initial number of regular or nutrient enhanced flies, r_1 and r_2 is number of flies consumed. To compare selectivity of the two fly types between the two prior diet treatments, I calculated α 's and 95% confidence intervals (CIs) for prior diet treatments for each prey type (regular and nutrient enhanced). The confidence interval indicates positive selection for a prey type when the CI is above the $\alpha = 0.5$ point, negative selection when the CI is below, and random or neutral when the CI overlaps with the no preference point ($\alpha = 0.5$). As a further test, the null hypothesis of no preference among the two prey quality types within a treatment (i.e. α nutrient enhanced = α regular = 0.5) was compared against the alternative of α nutrient enhanced $\neq \alpha$ regular using Hotelling's T^2 because of the inherent dependence in the α vector (i.e. $\Sigma \alpha_i = 1$)(Chesson 1983). Prey choice analyses were conducted in JMP 8.0 by implementing "Fit Model" and specifying personality as MANOVA (SAS Institute, Inc., Cary, IN, USA).

Results

There was no difference in the dry mass of flies reared on the different media (mean + 1 SE; regular: 0.334 + 0.012 mg; nutrient enhanced: 0.310 + 0.016 mg; $F_{1,56} = 2.16$, P = 0.15, block: $F_{12,56} = 1.53$, P = 0.14). However, the nutrient content of regular flies differed significantly from the nutrient enhanced flies ($F_{1,36} = 4.34$, P = 0.04, see Table S1 in appendix). Higher nitrogen content in the bodies of the nutrient enhanced flies (9.32 ± 0.14 %) as compared to regular flies (8.92 ± 0.22 %) translated into an estimated 11 % higher edible protein content in nutrient enhanced flies (ingestible protein content: regular = 0.075 mg per fly; nutrient enhanced = 0.083 mg per fly; Wilder *et al.* 2010).

Functional response: Prey quality and feeding history

Pardosa milvina exhibited a type II functional response on both prey quality types, where the proportion of flies killed decreased with increasing abundance (Table 1; Fig. 1a). Prey quality had no effect on the overall shape of the functional response, and there were no differences between regular and nutrient enhanced prey treatments in the proportion of prey killed (Table 1; Fig. 1a), or the calculated attack efficiencies or handling times (Table 2).

While the spiders assigned to treatments were not different in size or abdomen width prior to trials (see Table S2 in Appendix), consumption of prey differed by prey type and initial prey abundance (Table 3; Fig. 1c). Consumption, as measured by the change in abdomen width,

increased from 10 to 30 prey treatments and then leveled off (Table 3; Fig. 1c). Interestingly, the nutritional quality of prey provided to the spider affected consumption; the abdomen width of spiders foraging on regular prey increased more than those foraging on nutrient enhanced prey (Table 3; Fig. 1c)

The prior feeding history of *Pardosa milvina* affected their functional response. Spiders exhibited a type II functional response when fed on either prior diet treatment; however, spiders fed on a prior diet of nutrient enhanced prey killed consistently a greater proportion of prey than spiders fed the prior diet of regular prey (Table 1; Fig. 1b). Although the attack efficiency (α) was greater for spiders fed a prior nutrient enhance diet, this was not a statistically significant difference (Table 2). Spiders assigned to prior diet treatments were similar in size or abdomen width prior to trials (see Table S2), but consumption, indicated by change in abdomen width, increased during trials as the number of prey provided increased (Table 3; Fig. 1d). Consumption, similar to the prey type functional response experiment, increased at lower initial prey abundance and leveled off, but there was no effect of prior diet treatment on the final abdomen width of spiders (Table 3; Fig. 1d).

Prey choice: Prey quality and feeding history

When spiders were exposed to both low and nutrient enhanced flies were combined, there were no differences in the numbers of flies alive at the end of trials when flies were either nutrient enhanced red eyed (5.50 ± 1.05) or white eyed $(5.00 \pm 0.58; t = -0.42, df = 21, P = 0.67)$, which verifies that there was no difference in the vulnerability of the two mutants to spiders and no preference of particular eye colors by spiders that would have affected our results. Thus, the results were pooled, and these spiders that were fed a standard diet of two crickets preferred regular prey to nutrient enhanced prey (Hotelling's $T^2 = 12.96$, df = 6, P < 0.011; see Table S2 in Appendix). Although the overall pattern of selection was similar between spiders fed on prior diets of low or nutrient enhanced flies, spiders previously maintained on a regular diet had selectivity scores for both low and nutrient enhanced flies that overlapped the no preference line indicating no preference, or neutral selection (Hotelling's $T^2 = 1.41$, df = 14, P < 0.27, Fig. 2). The spiders fed a prior diet of nutrient enhanced flies exhibited significant positive selection for nutrient enhanced flies, and selected against regular flies (Hotelling's $T^2 = 6.90$, df = 13, P < 0.021, Fig. 2). Preference for quality types cannot be explained by fly size because flies raised

on the two media were similar in size (see Table S1), or spider size because *Pardosa milvina* were similar in size (carapace width) between treatments before the choice test (see Table S2). Likewise, body condition (abdomen width relative to carapace width) was similar prior to the choice test (see Table S2).

Discussion

Here I show that feeding history influences the functional response and prey preferences of generalist predators. Specifically, prey raised in different nutrient conditions affected consumption by spiders, and prior experience with prey differing in nutrient composition affected the proportion of prey that they killed across a range of prey densities. Both of these foraging shifts can alter the impact that these animals have in the food web. Surprisingly, our wolf spiders were also able to discriminate and select between fruit flies that differed only in their nutrient content, which potentially indicates that prey choice in field situations occurs on a finer scale than is typically considered. These results contribute to the growing evidence that nutrition can have sizeable consequences on trophic interactions, and that incorporating more detailed dietary information into models of food webs may provide insight into the structure and function of communities and ecosystems (Raubenheimer *et al.* 2009; Schmitz *et al.* 2010).

While prey quality did not affect the number of prey that *P*, *milvina* killed, it did influence the amount of those prey that was consumed by spiders, as evidenced by the change in abdomen size (Figs 1a and 2a). The protein leverage hypothesis predicts that consumers will continue to feed until they reach some target protein intake level (Sørensen *et al.* 2008; Raubenheimer *et al.* 2009). Thus, the higher consumption of the regular prey that contained less nitrogen (and by extension less protein) than our nutrient enhanced flies may be due to the spider's drive to cross that protein threshold. The crickets provided to these spiders in the weeks prior to testing contain relatively high levels of both protein and lipid (Wilder *et al.* 2010) and so if the animals were, in any way, attempting to match their recent protein intake they would have to extract much more material from the regular flies.

The recent feeding history of *P. milvina* shifted the intercept of the functional response generated for nutrient enhanced flies but had no impact on our measure of consumption (Figs 1b and 2b). Although I anticipated that animals maintained on regular prey for three weeks would have higher kill rates when confronted with an abundance of nutrient enhanced prey items, the

opposite occurred. Interestingly, in a mating study, female P. milvina fed nutrient enhanced fruit flies were more aggressive toward males both before and after mating (Wilder and Rypstra 2008). These effects are likely to be due either to diet effects on the ability of a spider to kill prey or some change in their inherent aggression level (Mayntz and Toft 2006; Wilder and Rypstra 2008). However, if their foraging ability was altered by diet, I would have expected the estimate of handling time in the functional response to be affected, and although the attack rate on prey did not differ for the prior diet treatment is was higher, which is consistent with the difference in proportion of prey killed between the two diet treatments (Table 2). Thus, it seems most likely that *P. milvina* were simply more aggressive and more motivated to capture prev when they were fed a nutrient enhanced diet. Little is known of the specific nutritional requirements for spiders or of other nutritional differences between the flies I used beyond the protein values reported here or reported as other nutrients (Wilder et al. 2009). For a variety of vertebrates, the interactions among the hormones and neurotransmitters that control aggression are well documented, and shifts in aggressive behavior can be generated by providing them with diets essential nutrients needed for metabolic signals (Simon et al. 2004; Bosch et al. 2007; Poletto et al. 2010). Ness et al. (2009) show that plants may stimulate predation on herbivores by supplying ants with nectar high in carbohydrates, indicating that nutrient limitation may aid in herbivore control. On the other hand, for Argentine ants, *Linepithema humile*, dietary carbohydrate levels affected aggression but the availability of insect prey, which presumably would supply more protein, did not (Grover et al. 2007). Hence, although I know that our flies differed in protein content, I do not know if this particular difference can account for the shift in foraging observed. Clearly further research is needed to determine how specific components of the diet affects the physiology of the spiders and ultimately alters their predatory response.

The functional response has been used extensively as a tool to assess the potential of natural enemies to control pest populations (Riechert and Lockley 1984; Barlow and Goldson 1993; Lester *et al.* 1999; Pekár 2005). Our results suggest that recent feeding history influences the functional response in non-intuitive ways. For example, I might have assumed that a poorly fed predator would have had the largest impact on potential pest populations but I discovered that well-fed predators had higher predation rates (Fig. 1a). Thus, small nutritional differences in the alternative prey available to the spiders before a pest outbreak could alter their efficacy of agents of biological control. Similarly, if the spiders happen to focus their foraging effort on

poor quality pest insects early in an outbreak, their kill rates may actually decrease before the populations are under control.

In general, spiders are not thought to be choosy foragers; they have been reported to consume from as many as 14 different insect orders (Riechert 1992). However, spiders in our study were able to discriminate between members of the same species of prey that differed in nutritional content. Although I was only able to measure the nitrogen and carbon levels of the two prey types, other chemicals were likely in different concentrations as well (i.e. vitamins and minerals; Wilder et al. 2009). That these spiders exhibited active choice driven by differences in body composition of the prey may indicate that these other nutrients are important as well. Future studies will be able to distinguish which of the other nutrients is limiting, but my current work shows that these spiders discriminate among prey based on nutrient content, which has yet to be incorporated into a general understanding of prey choice. Furthermore, choice was affected by prior diet, which complicates prey selection because prior experience may be important in determining future prey use (Fig. 3). In other studies, flies, similar to those used in our study, were of such low amounts of essential nutrients that they were unable to support the complete development another wolf spider species (Mayntz and Toft 2001). On the other hand, spiders with experience feeding on nutrient enhanced prey were likely in better nutritional condition and, as such, could be more selective in their choice of food (and able to express a preference for nutrient enhanced flies).

Although my study showed that spiders were able to choose between fruit flies that have different nutrients, it is not clear how they could make such a high-resolution choice. Previous work has shown that some wolf spiders will select familiar prey (Persons and Rypstra 2000) and so the results here may simply be an offshoot of that decision making process. However, only the spiders fed high nutrient prey selected familiar prey; those that had been maintained on regular fruit flies showed no preference (Fig. 3). Added to that, *P. milvina* is highly attuned to its chemical environment and able to discriminate based on changes in prey (Hoefler *et al.* 2002) and predator cues (Rypstra and Persons 2006; Bell *et al.* 2006). The chemosensitivity of *P. milvina* potentially allowed them to detect subtle differences in chemical signature emitted by prey that differed in quality and to use that information to select prey based on their current nutritional needs.

Although it is not currently known how spiders or other predators regulate or prioritize nutritional needs, our results show that prior experience to differing in nutrient content altered both the functional response and prey preference of a common generalist arthropod predator. While much attention has been focused on the effects of nutrients on organisms with in food webs (Fagan and Denno 2004, Fagan et al. 2004, Bukovinszky et al. 2008), the influence of prey nutrient quality has yet to be integrated into the metrics used to quantify foraging such as the functional response (Abrams 2010). Prey size is an important feature of the predator-prey interaction and is receiving support for scaling predator-prey interactions (Brose 2010). These results suggest that if prey size and prey species identity are controlled, then current and prior experience with prey differing in body nutrient composition are important conditions influencing the frequency of predation and predator consumption of prey. The predators in our study were able to make fine-scale discrimination between fruit fly prey based on their nutrient content. In the field this may translate into switching between prey or allocation of time to forage on prey with specific nutrient signatures, which may complicate the functional response (Abrams 1987), and potentially lead to indirect effects on other prey in the system (i.e. apparent competition; Holt 1977). Future studies should address the role of prey nutrient status in reshaping trophic interactions and community composition in the field. This study also shows that more studies are needed to understand the degree to which the predatory potential of important natural enemies is driven by prioritization of nutrient intake, and prior experience with fluctuation in prey quality.

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Table 1. Results of logistic regression used to determine the type of functional response of each functional response experiment, and to test for differences between either prey type or prior diet in the proportion of prey killed by the wolf spider, *Pardosa milvina*. Estimates of coefficients and standard error (SE) are presented for initial prey abundance, N_o , quadratic term for initial prey abundance, N_o^2 , and prey type or diet which represent the indicator variable in the model to test for differences between either prey type or prior diet treatments (n= 8-10 per treatment combination for each experiment).

	Estimate	SE	X^2	Р
Prey type				
Intercept	3.368	0.402	81.88	< 0.0001
N_{0}	-0.119	0.024	25.08	< 0.0001
N_0^2	0.001	0.000	12.83	0.0003
Prey type	0.076	0.043	3.16	0.0756
Prior Diet				
Intercept	2.805	0.315	79.28	< 0.0001
N_{0}	-0.072	0.019	13.89	0.0002
N_0^2	0.001	0.000	6.00	0.0143
Diet	0.201	0.039	26.79	< 0.0001

Table 2. Estimates of type II functional response parameters for the two functional response experiments using the wolf spider, *Pardosa milvina*. Current prey type was the first experiment where abundance of each prey quality type was manipulated, and prior diet represents parameter estimates from the second experiment where spiders were fed on either regular or nutrient enhanced prey for a three-week period prior to the functional response. Values in parentheses indicate $\pm 1SE$ around estimates.

	Regular	Nutrient enhanced	df	t	Р
Prey type					
Attack constant (<i>a</i>)	0.110 (0.037)	0.096 (0.048)	1, 96	0.03	0.82
Handling time (T_h)	0.486 (0.128)	0.369 (0.189)	1, 96	0.51	0•61
Prior diet					
Attack constant (<i>a</i>)	0.082 (0.017)	0.129 (0.036)	1, 98	1.18	0.24
Handling time (T_h)	0.326 (0.103)	0.398 (0.132)	1, 98	0•43	0.67

Table 3. Results from ANOVA used to determine the effect of prey type or prior diet on prey consumption, as measured by natural logarithm transformed change in abdomen width of *Pardosa milvina* over the 24 hr foraging period. Prey treatment represents the regular or nutrient enhanced *Drosophila melanogaster*.

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Source	df	SS	F	P
Prey type				
Prey treatment	1	0.23	15.21	0.0002
Initial prey abundance (N_0)	4	0.61	10.16	< 0.0001
Interaction	4	0.03	0.54	0.70
Residual	96	1.34		
Prior diet				
Prey treatment	1	0.02	0.64	0.43
Initial prey abundance (N_0)	4	0.74	7.22	< 0.0001
Interaction	4	0.12	1.19	0.32
Residual	98	2.99		

Figure 1. Effect of prey abundance on mean proportion of prey types killed by *Pardosa milvina*, left hand panels (A) prey type and (B) prior diet. Effects of prey abundance on mean consumption, right hand panels (C) prey type and (D) prior diet. Regular prey types or diets are represented with open symbols or bars, and filled represent nutrient enhanced treatments. Values indicated by symbols represent the mean proprotion (\pm 1SE), n= 8-10 per treatment combination. The lines for panels (A) and (B) represent the logistic regression predicted values, where dashed lines are the regular prey or regular prior diet and the solid line represents the enhanced prey or enhanced diet.



Figure 2. Prey selectivity by *Pardosa milvina* (mean and 95% CI) for low (white bars) or nutrient enhanced flies (shaded bars) when spiders were fed a prior diet of regular or nutrient enhanced flies for a period of three weeks prior to selectivity tests. Values on the ordinate represent the mean α preference scores (Chesson 1983). The dashed horizontal line at $\alpha = 0.50$ represents random prey consumption. When the 95% CI is below the horizontal line, this indicates negative selection; when the 95% CI is above the line, there is positive selection.


Appendix

Table S1. Means (\pm 1 SE) percent nutrient content and statistical tests comparing the nutrientcontent of the regular *Drosophila melanogaster* on the standard *Drosophila* media, and nutrientenhanced flies reared on dogfood-supplemented medium.

Variable	Regular	Nutrient enhanced	F _{1,36}	Р
Percent C	51.81±0.21	51.62±0.14	0.53	0.47
Percent N	8.92 ± 0.22	9.32±0.14	4.34	0.04
C:N	6.79±0.11	6.48±0.10	4.52	0.04

Source	Mean ± 1SE	df	F	Р
Current Prey type				
Spider size		1, 96	0.25	0.62
Regular	2.13 ± 0.03			
Nutrient enhanced	2.14 ± 0.03			
Body condition				
Carapace (covariate)		1,96	27.61	< 0.0001
Treatment		1,96	0.29	0.59
Regular	1.89 ± 0.04			
Nutrient enhanced	1.86 ± 0.04			
Prior diet				
Spider size		1, 98	1.64	0.20
Regular	2.16 ± 0.02			
Nutrient enhanced	2.13 ± 0.02			
Body condition				
Carapace (covariate)		1, 98	13.56	0.0003
Treatment		1, 98	0.70	0.41
Regular	2.03 ± 0.03			
Nutrient enhanced	1.99 ± 0.03			
Prey choice				
Spider size		1, 41	1.60	0.22
Regular	2.09 ± 0.04			
Nutrient enhanced	2.01 ± 0.01			
Body condition				
Carapace (covariate)		1, 24	5.39	0.03
Treatment		1, 24	1.14	0.30
Regular	1.98 ± 0.07			
Nutrient enhanced	2.00 ± 0.08			

Table S2. Results from analyses to determine the equivalence in size (ANOVA; carapace width, mm), and body condition (ANCOVA; abdomen width, mm, relative to carapace width, mm; multiple regression model) of spiders, *Pardosa milvina*, assigned to treatments for the functional response and prey choice experiments.

Table S3. Data from the preference experiment where the feeding history of spiders was manipulated (diet treatment) over a three-week period, then preference for regular or nutrient enhanced (regular fly medium enhanced with crushed dogfood) prey was analyzed. Reported here are the mean (\pm 1SE) of flies of each quality type remaining alive following the 24-hr foraging period (N = total number of replicates prior to exclusion based on prey depletion where the number remaining alive at the end was n<10).

Diet Treatment	N	Regular alive	Nutrient enhanced alive
Cricket fed (mutant control)	22	1.56(0.31)	5.23(0.56)
Regular	20	7.92(1.38)	5.54 (1.04)
Nutrient enhanced	21	8.75(1.39)	5.17(0.89)

GENERAL CONCLUSIONS

Synthesis of theoretical studies has determined that understanding individual animal foraging behaviors is essential to predicting the strength of potential food web links and interactions between species in ecological communities (see review Beckerman et al. 2006). Therefore, to model communities requires experiments that acquire relevant information on foraging behaviors that can be used to estimate and scale parameters to characterize more complex communities. In this dissertation, I reported a series of experiments that explored factors that are predicted to influence patch use and diet selection of generalist predators from multiple vantage points. Specifically, I explored classic aspects of how researchers view the response of predators to prey (i.e. through measuring abundance response) and functional response (i.e. feeding rate changes or eating more prey when more are available), and used a relatively new approach to track predation in natural settings using molecular analysis of gut contents. Pardosa displayed characteristics of an adaptively foraging predator in choosing between habitats that required this spider to make trade-offs and modify its consumption of prey. These experiments provide clear evidence of decision making in a generalist arthropod predator in response to a wide variety of environmental challenges that appear to influence their trophic interactions through shifts in space use and prey selection.

Space use

Variation in foraging behavior may be due to the heterogeneity of both resource availability and suitable habitat (Brown 1988; Perry and Pianka 1997). Habitat structure, in particular, is shown to have very strong effects on generalist arthropod predators (Langellotto *et al.* 2005). Structural features can temper intraguild predation (Finke and Denno 2006), leading to higher predator densities, which demonstrates that changes in habitat use driven by prey and structure generate complex and interactive impacts of bottom-up and top-down forces in food webs. My studies characterize the wandering spider, *Pardosa milvina*, as one that actively forages and makes patch choice decisions based on multiple patch quality indicators (Rypstra *et al.* 2007; Chapter 1, Chapter 2). For example, *P. milvina* prefers to forage in areas containing less risk (Rypstra *et al.* 2007) and habitat structure had positive effects on their colonization of patches (Fig. 1a), which was driven by their foraging ability and the indirect effect of reduction in risk (Fig. 1b). Risk was realized as abundance of intraguild predators and cannibalism (Figure 1c,e). Therefore, although habitat structures had a positive effect on prey abundance (Chapter 1 and Figure 1d) by providing space and organic material, the effects of risk and foraging ability trumped the importance of prey abundance in determining a foraging site. However, when I removed risk from the equation (Chapter 2), their tendency to remain in a patch and forage, was motivated by prey availability more so than interference between conspecifics (Figure 1e, f). Combined these results indicate that *Pardosa* is sensitive to the texture of the environment (i.e. microsite structure) and the resources present in potential foraging sites and makes trade-offs to forage in sites lower in risk (both predation risk and interference), and higher in prey abundance.

Prey selection

Observational studies indicate that spiders consume a wide variety of prey species (see review Nyffeler and Benz 1994); therefore, the prevailing historical hypothesis was that spiders are true generalists and eat anything available within a certain size range (Turnbull 1973; Reichert 1992; Wise 1993). Many hypotheses have been proposed to explain prey selection including prey quality, diet mixing, aversion (Toft 1999), and more broadly for generalist predators, density dependent selection (Sherrat and Harvey 1993). There is evidence to support each of these hypotheses (Toft and Wise 1999; Harwood *et al.* 2004; Mayntz *et al.* 2005) or counter evidence for aversion (Toft and Wise 1999), which suggests that there may not be a generalizable prey selection mechanism in diverse taxa such as spiders. This means that simply lumping all spiders into one trophic level may not be the best way to model their ecological role, and using guilds (Uetz 1999) or functional groups (Flynn *et al.* 2009) is a better approach when individual based knowledge of prey selection is lacking.

Prey traits likely have important impacts on prey choice and the functional response of predators (Brose 2010; Loeuille 2010). The most common trait currently used in adaptive foraging models is prey size (see review Brose 2010). In general, predators tend to feed on prey that are smaller, and therefore, larger predators have the ability to capture a broader range of prey sizes (Cohen *et al.* 1993). Although the diets of spiders are shown to be restricted by size (Enders 1975; Erickson and Morse 1997), evidence is building in support of active prey selection based on other prey traits (Toft 1999). Chapters 3 and 4 of my dissertation provide further support for active prey selection by showing that *Pardosa milvina*, in interesting ways was sensitive to abundance of prey, and to nutrient content of prey. When exposed to a diversity of

prey species, these spiders appeared to increase uptake of flies with increase in abundance, but not in direct proportion to relative abundance (Figure 1c). Most surprisingly, although the functional response suggests captureability of prey was not influenced by prey nutrient content, *Pardosa milvina*'s nutritional experience influenced prey choice and the proportion of prey killed (Figure 1f,c). These results indicate that shifts in diet in *Pardosa milvina* are potentially less driven by abundance alone, and are likely triggered by internal condition, which determines preference for and foraging rate of available prey types.

Foraging behavior continues to intrigue and challenge ecologists (Beckerman *et al.* 2006, Stephens *et al.* 2007, Abrams 2010). Documenting behavioral changes and implementing those changes into how food webs and ecological communities function is important for examining current model predictions (Loeuille 2010). However, assessing changes in foraging behavior in natural settings is often difficult, especially in cryptic arthropod predators (Nyffeler *et al.* 1994; Greenstone 1996). Molecular techniques are continuing to provide an efficient avenue to characterize feeding links in natural settings (Symondson 2002; Sheppard and Harwood 2005), and in my studies showed that predation on one prey link was not strictly driven by abundance of prey. Future research in agricultural systems containing *Pardosa* should target more food web links to identify with higher resolution the full spectrum of prey used, and the conditions determining the differential reliance on prey species. For example, recent PCR-based analyses are capable of characterizing the diets of extremely polyphagous predators in a variety of taxa (Sheppard and Harwood 2005; Clare *et al.* 2009). One study in particular discovered that the diets of bats were composed of as many as 127 species of insects (Clare *et al.* 2009).

Although molecular techniques provide rich information about predation history in food webs, what appears to be lacking is the development of a clear integration of molecular based predation data with food web models or community models (Johnson *et al.* 2009). Models incorporating molecular data have surfaced in attempts to link predation frequency to the functional response, but the predictive power is still quite low, in part due to the complexity of incorporating the decay of the molecular marker in the guts, what prey abundance estimates really tell us about the predator's attack rates on prey, and few developments have surfaced in the literature since Naranjo and Hagler (2001). Hence, currently, most molecular analysis of feeding links is qualitative in nature and provides information on linkage between species without quantitative integration of interaction strength between species.

To get a more quantitative view of food web interactions requires incorporation of the magnitude of potential and realized interactions in food webs (May 1983). Quantitative analysis of trophic interactions requires gathering data on link density determined by the effective number of each prey species eaten (or biomass transfer or energy transfer) over time and space (Banašek-Richter *et al.* 2009). Secondly, quantitative food web analyses use these data to present information on the linkage between species in addition to the strength of interactions or magnitude (Paine 1988). This is difficult in arthropod communities because it is challenging to get accurate estimates of prey eaten per some time value or the actual biomass eaten. Furthermore, currently, the decay curves of PCR and monoclonal antibodies are used to validate the use of a particular probe, but these data, with appropriate experimental conditions (i.e. gradients of the number of prey eaten or size of prey), will prove useful in calibrating interactions between species. In addition, molecular tools for studying predation are advancing rapidly. In the near future molecular data will be used in modeling population dynamics in food webs, and there are examples of these techniques used in a quantitative analysis of food webs (see Kaartinen *et al.* 2010).

Summary and future research

There is still much to understand about the foraging behavior of common spiders such as those in the genus *Pardosa*. For instance, learning has the potential to change the dynamics between predator and prey by introducing a lag time in the response to prey where predators must first learn to associate specific prey behavior (Abrams 2010). Although it does not appear that spiders in the genus *Pardosa* can learn to avoid toxic prey (Toft and Wise 1999), this does not rule out the plausibility that these spiders can learn. Rearing experiments in *Pardosa* suggest that prior experience with differing levels of habitat complexity impacted their ability to capture prey in those environments (Unpublished data Folz), which suggests a learning response to the structural elements present. In addition, in Chapter 4 the prior experience with prey that differed in nutrient content does suggest an ability to gauge prey nutrient content, but further research is needed to understand how and what aspects they cue into. For instance, prey quality may alter the perception of prey availability (Fig. 1h) because although prey abundance influences the functional response, selectivity for specific prey nutrients lessens the possible prey pool. Further study should link prey quality in the field to prey use and using a mixed prey

environment with known nutrient history of the predator, assess switching between prey types. Furthermore, more research is needed into how other prey traits influence diet selection. In Chapter 3, *Pardosa* appeared to avoid leafhoppers and these prey as compared to others in the manipulated community potentially differ in their defenses (Denno *et al.* 2003), and/or morphology that make them less palatable or more difficult to attack. In general, incorporating more prey traits into our understanding of interactions between *Pardosa* and its prey, and other generalists within complex food webs will lead to a better understanding of specific behavioral and morphological traits influencing the magnitude of interaction between trophic linkages. Further experimental research on prey selection in arthropod communities using PCR-based analysis of food web links also is required to understand the consequences of prey selection on community structure. In particular, combining the use of molecular probes with quantitative food web analyses to determine the magnitude of food web links will reveal the roles of species in complex food webs.

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Figure 1. Factors measured that influenced the foraging behavior of *Pardosa milvina*. Arrows indicate the connection between various factors and *Pardosa milvina*. The weight of the arrow represents a qualitative interpretation of the importance of the link to altering either space use or prey selection. The solid arrows represent direct effects of factors and dashed arrows indicate indirect effects mediated through the response of these predators to an aspect of the environment, (i.e habitat structure, or to a prey trait, nutrients). Letters on arrows were used in the body of the general conclusions text to emphasize specific findings in this dissertation.

