Insect. Soc. 54 (2007) 219 – 224
0020-1812/07/030219-6
DOI 10.1007/s00040-007-0933-x
© Birkhäuser Verlag, Basel, 2007

Insectes Sociaux

Research article

Protein marking reveals predation on termites by the woodland ant, *Aphaenogaster rudis*

G. Buczkowski and G. Bennett

Department of Entomology, 901 W. State St., Purdue University, West Lafayette, IN 47907, USA, e-mail: *gbuczkow@purdue.edu*

Received 19 January 2007; revised 23 March 2007; accepted 26 March 2007.
Published Online First 20 April 2007

Abstract. Subterranean termites provide a major potential food source for forest-dwelling ants, yet the interactions between ants and termites are seldom investigated largely due to the cryptic nature of both the predator and the prey. We used protein marking (rabbit immunoglobin protein, IgG) and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) to examine the trophic interactions between the woodland ant, *Aphaenogaster rudis* (Emery) and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). We marked the prey by feeding the termites paper treated with a solution of rabbit immunoglobin protein (IgG). Subsequently, we offered live, IgG-fed termites to ant colonies and monitored the intracolony distribution of IgG-marked prey. Laboratory experiments on the distribution of protein-marked termite prey in colonies of *A. rudis* revealed that all castes and developmental stages receive termite prey within 24 h. In field experiments, live, protein-marked termites were offered to foraging ants. Following predation, the marker was recovered from the ants, demonstrating that *A. rudis* preys on *R. flavipes* under field conditions. Our results provide a unique picture of the trophic-level interactions between predatory ants and subterranean termites. Furthermore, we show that protein markers are highly suitable to track trophic interactions between predators and prey, especially when observing elusive animals with cryptic food-web ecology.

Keywords: *Aphaenogaster rudis*, *Reticulitermes flavipes,* predation, prey-predator interactions, protein marking.

Introduction

Animal ethology and ecology studies often involve experiments in conditions that may preclude visual observations. This is especially true when observing the trophic ecology of small and/or elusive animals with cryptic behavior. While vertebrate food webs often involve visible predator-prey interactions, which makes field observations relatively straightforward, invertebrate food webs are often substantially more difficult to document. To accurately asses the trophic interactions between invertebrate predators and prey, an efficient marker is required. Current approaches to study predator-prey interactions in invertebrates may involve radioisotope labeling (Showler et al., 1988; Breene and Sterling, 1988), stable isotopes (e.g. ^{15}N, Nienstedt and Poehling, 2004), visual identification of gut and/or fecal samples (Sunderland, 1988; Ingerson-Mahar, 2002), serological tests that utilize either protein markers (Hagler and Durand, 1994; reviewed in Hagler and Jackson, 2001; Hagler, 2006) or monoclonal antibodies (Hagler et al., 1992; Harwood et al., 2004; reviewed in Sheppard and Harwood, 2005), or various DNA-based techniques (Augusti et al., 2003; reviewed in Symondson, 2002; Harper et al., 2005; reviewed in Sheppard and Harwood, 2005). Despite the availability of these techniques, few studies have examined trophic interactions in invertebrate food-webs, especially under field conditions. Here, we demonstrate the utility of protein marking to track and quantify the trophic connection between a generalist insect predator and its insect prey. Specifically, we examine predation by the woodland ant, *Aphaenogaster rudis* (Emery) on the eastern subterranean termite, *Reticulitermes flavipes* (Kollar).

Ants (Hymenoptera) and termites (Isoptera) have been co-evolving for over 100 million years (Hölldobler and Wilson, 1990) and the great majority of ant-termite

interactions are highly antagonistic, with the soft-bodied termites usually losing the battle to the heavily armed and sclerotized ants. Many species of ants prey on termites opportunistically and several ant genera are specialized termite predators (*Leptogenys*, *Termitopone*, and *Megaponera*; e.g. Sheppe, 1970; Deligne et al., 1981; Hölldobler and Wilson, 1990). Despite the evolution of a variety of defensive mechanisms, most notably the soldier caste (Deligne et al., 1981; Mill, 1983), termites remain an attractive target for predatory ants. Termites are attractive prey because their colonies, if viewed as food items, possess numerous characteristics important to foraging ants. First, termite colonies provide an exceptionally concentrated and protein-rich food source and are thus a high value prey (Wood and Sands, 1978). As such, they fulfill the predictions of the foraging theory (Carroll and Janzen, 1973; Oster and Wilson, 1978) which suggests that foraging costs should be minimized in order to optimize the yield and thus the net energy gain. Second, termite colonies are relatively sessile (confined within the bounds of the log) and stable through time. Stable food sources can be more efficiently exploited by reducing travel distances and travel time (Hölldobler and Lumsden, 1980; McIver, 1991; Davidson, 1997). Termite colonies are also highly clumped, thus allowing efficient prey discovery and retrieval (Davidson 1977, Traniello, 1983; Pasteels et al., 1987). Other benefits of foraging on termite nests, especially if the ants and the termites are nesting in the same log, may include: reduced exposure to natural enemies and predators, decreased likelihood for workers to become disoriented, ability to maintain optimal scout-to-recruit ratios, no need to time food appearance with labor availability, reduced need to maintain and defend an extensive foraging range, high longevity of unharvested food items, and protection of the ant's living space from damage by the termites which often feed on logs inhabited by ant colonies.

While the behavioral interactions between ants and termites have been examined in numerous studies (Cornelius and Grace, 1996; Longhurst et al., 1979; Leponce et al., 1999) there are no studies to document the degree to which ants may prey on termites under field conditions and it is unknown how predatory ants may utilize termite prey. In this study, we examined predation by the woodland ant, *Aphaenogaster rudis* (Emery) on the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). We chose *A. rudis* and *R. flavipes* based on their high relative abundance in mixed hardwood forests, similar nesting requirements and thus high propensity to interact, previous indications that *A. rudis* is a predatory ant (Southerland, 1988), and our personal observations which indicated that *A rudis* frequently inhabits logs and tree stumps colonized by *R. flavipes* with apparently little separation between the species. *A. rudis* is widely distributed throughout deciduous forests in eastern North America (Lynch, 1981; Herbers, 1989) and nests are often found at high densities. For example, Talbot (1957) found 1.85 *A. rudis* colonies per square meter in a Missouri forest. The eastern subterranean termite, *R. flavipes* is the most common and the most widely distributed subterranean termite in the eastern United States (Nutting, 1990). Despite the abundance of *A. rudis* and *R. flavipes* in temperate forests and their apparent proximity, little is known about possible interactions between the two species. This partly results from the secretive nature of termites which usually remain belowground where they form diffuse colonies composed of multiple nests and feeding sites connected by subterranean trails. The ants, too, remain cryptic under or within logs, and interactions between *A. rudis* and its prey are not easily observed under field situations.

To examine the trophic interactions between *A. rudis* and *R. flavipes* we utilized protein marking (reviewed in Hagler and Jackson, 2001) and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA; Hagler, 1997) to track the fate of termite prey in ant colonies. Previously, protein marking has proven an effective tool to study the flow of food in colonies of honeybees (DeGrandi-Hoffman and Hagler, 2000), ants (Buczkowski and Bennett, 2006), and termites (Buczkowski et al., 2007). Our objectives were to: (i) examine the suitability of the protein marker to study trophic-level interactions, (ii) use protein marking to examine the distribution of *R. flavipes* prey in laboratory colonies of *A. rudis*, and (iii) use protein marking to verify predation on *R. flavipes* by *A. rudis* in the field.

Materials and methods

Collection and maintenance of insect colonies

Colonies of the woodland ant, *Aphaenogaster rudis* (Emery) and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) were collected in a mature beech-oak-hickory forest at the Horticulture Park on the campus of Purdue University, West Lafayette, IN. Ant colonies collected from rotting logs of similar size were established in Fluon™-coated plastic trays provided with a moist plaster nest. Each laboratory colony was censused to determine the queen status and the number of workers and larvae. *A. rudis* colonies include a single reproductive queen and up to 2000 workers (Headley, 1949). All colonies were collected during May-July 2006. Ant specimens were identified based on worker external morphology (Creighton, 1950). Subsequently, colonies were maintained in debris-free trays and reared on 20% sucrose solution *ad libidum*, minced crickets, and artificial diet (Bhatkar and Whitcomb, 1970) twice a week. Colonies were maintained at 25 ± 2°C, 60 ± 10% RH, and 14:10 L:D cycle. Termite colonies were collected by trapping within cardboard rolls inserted into the ground and/or cavities of live, termite- infested trees. Colonies were brought into the laboratory and allowed to migrate into plastic containers with cellulose powder, moistened pine wood, and laboratory paper towels provided as food and harborage. Species identity was verified by external morphology of soldiers (Nutting, 1990). Colonies were maintained at 25–27°C, > 80% RH, and in constant darkness. Water was added to rearing containers as needed.

Intracolony distribution of prey in laboratory colonies of A. rudis

We examined the intracolony distribution of termite prey in *A. rudis* by providing the ants with termites that previously fed on IgG-treated paper and subsequently detecting the protein marker in the ants by using an enzyme linked immunosorbent assay (ELISA). To prepare the IgG-marked prey, a group of 50 termite workers was placed in a 9.5 cm diameter Petri dish containing 10 g of moist sand:vermiculite mixture and one 3.5 cm diameter piece of paper towel treated with 50 μl of 5 mg/ml solution of rabbit IgG. The termites fed on the IgG-treated paper for 24 h. Previous research showed that this method results in 100% of termites testing positive for the marker and that the termites retained the marker internally without becoming contaminated on the cuticle (Buczkowski et al., 2007). A queenright ant colony containing 200 workers and approximately 100 brood of various stages was placed in a 50 by 38 by 7 cm high plastic, Fluon™-coated tray and allowed to colonize a moist plaster nest. The ants were allowed to acclimate to the nest for 1 wk while provided with food, as described above. After the acclimation period, the colony was starved for 24 h and was subsequently provided with 15 live termites that previously fed on IgG-treated paper. The intracolony distribution of the prey was examined 24 h later by randomly sampling 15 workers, 15 large larvae (average weight, 9.2 ± 0.06 mg, $n = 20$), 15 small larvae (average weight, 1.2 ± 0.05 mg, $n = 20$), and the queen and subjecting all individuals to ELISA (see below) to determine the amount of protein marker acquired. The distribution of prey was also examined at 48 and 72 h using new queenright colonies and 4 replicates were performed for each time point.

Intracolony distribution of prey in field colonies of A. rudis

To determine whether *A. rudis* preys on *R. flavipes* under field conditions we provided field colonies of *A. rudis* with the opportunity to prey on termites. Colonies of *A. rudis* were located at the Horticulture Park on the campus of Purdue University, West Lafayette, IN by visually inspecting logs for foraging ants. Whenever foraging ants were found, the log was baited with several dead termites. Ants carrying termites were then traced back to the nest and the location of the nest was marked. Subsequently, each experimental colony ($n = 5$) was provided with a weigh boat containing 15 live termite workers that previously fed on IgG-treated paper (see methods above). The dish was placed approximately 1 m away from the nest and was continuously observed to make sure that all termites were retrieved by *A. rudis* and not competing species. Fifteen workers, 15 small larvae, and 15 large larvae were collected from each colony 48 h later, returned to the laboratory, and analyzed for the presence of the marker using sandwich ELISA. The 48 h sampling time was chosen based on the results on laboratory assays which indicated that the largest proportion of individuals tests positive for the marker at 48 h. All tests were carried out during the peak of colony activity (June-August; Headley, 1949).

The ELISA procedure

Sandwich ELISA was performed on individual ant samples using previously described techniques (Hagler, 1997; Buczkowski et al., 2007). Ant samples were individually homogenized in 150 μL phosphate buffered saline (pH = 7.4) and assayed for the presence of the rabbit immunoglobin protein. Each well of a 96-well microplate was coated with 100 μL of anti-rabbit IgG (developed in goat) (Sigma Chemical Co., St. Louis, MO) diluted 1:500 in distilled water and incubated for 2 h at 4°C. After incubation, the primary antibody was discarded and 310 μL of 1% non-fat dry milk (Bio-Rad Laboratories, Hercules, CA) in distilled water was added to each well to block any remaining non-specific binding sites. After 30 min. incubation at 26°C the milk was discarded. Ant samples were vortexed, added to each well, and incubated for 1 h at 26°C. The samples were then discarded and each well was washed 3 times with PBS Tween 20 (0.05%) and 2 times with PBS. Anti-rabbit IgG conjugated to horseradish peroxidase (50 μL) diluted 1:1,000 in 1% non-fat milk was added to each well and incubated at 26°C for 1 h. All wells were washed again as above and 50 μL of TMB HRP substrate (BioFX Laboratories, Owings Mills, MD) was added to each well and incubated for 30 min. Samples were analyzed on a Beckman Coulter AD 340 Absorbance Detector set at 620 nm. The mean (± SE) optical density value and the percentage of samples scoring positive for rabbit protein were determined. Six negative controls (ants never exposed to termites fed rabbit IgG) and 6 blanks (PBS buffer only) were run on each plate.

Statistical analysis

For immunomarking experiments, the samples were scored positive for the presence of the protein marker if the ELISA optical density value exceeded the mean negative control value by three standard deviations (Hagler, 1997). Based on results of preliminary tests, the value for the positive-negative threshold (mean value for negative controls ± 3 S.D.) was determined to be 0.06. The percentage of samples testing positive for the IgG protein was tabulated by first calculating the percent of individuals testing positive within a replicate and then averaging across replicates. Percentage data were arcsine transformed to stabilize the variance. ANOVA tests were conducted to determine the significance of developmental stage on the spread of the marker. This was accomplished by using the PROC GLM procedure in SAS 8.1 (SAS, 2002), followed by post-hoc Tukey's HSD tests to separate the means for statistical differences. For all immunomarking experiments the results are expressed as both: (1) the mean percentage of individuals testing positive, and (2) the mean optical density (OD). All mean OD values are an average of all individuals used in the test, not just those testing positive.

Results

Intracolony distribution of prey in laboratory colonies of A. rudis

In laboratory feeding experiments, *A. rudis* immediately attacked termite prey and delivered envenomed termites to the nest. The prey were then dismembered and fed to the larvae and we frequently observed 2–3 larvae feeding on a single termite carcass. After about 24 h, few termite remains were still visible indicating that all prey were completely consumed by the colony. At 24 h, 47 ± 8% of *A. rudis* workers, 33 ± 8% of small larvae, 43 ± 11% of large larvae, and 75 ± 25% of queens tested positive for termite remains (Table 1). These values increased significantly at 48 h for both workers and larvae indicating that these stages further accumulate the marker by feeding directly on the prey and/or receive prey remains by trophallaxis with other individuals. At 72 h, the percentage of ants testing positive started to decline suggesting that the marker may have been metabolized and/or excreted.

Intracolony distribution of prey in field colonies of A. rudis

Whenever an *A. rudis* scout located a dish with termite prey, it harvested a single termite and carried it back to the colony. More workers were then recruited to the dish

Table 1. Mean percentage ± SE of *A. rudis* testing positive for rabbit IgG protein after preying on protein-marked termites fed IgG-treated paper. Mean optical density (OD) values ± SE for individuals testing positive are given in parentheses. Means followed by the same letter are not significantly different by Tukey's HSD test ($P \leq 0.05$). First letter indicates within row comparisons, second within column comparisons.

Caste/developmental stage	Mean % positive		
	24 h	48 h	72 h
workers	47 ± 8% b, b (0.30 ± 0.05)	77 ± 7% a, a (0.27 ± 0.02)	70 ± 4% ab, ab (0.26 ± 0.03)
small larvae	33 ± 8% b, b (0.25 ± 0.06)	62 ± 3% a, b (0.21 ± 0.02)	45 ± 10% b, c (0.19 ± 0.04)
large larvae	43 ± 11% a, b (0.20 ± 0.04)	72 ± 7% b, ab (0.26 ± 0.02)	52 ± 6% a, bc (0.20 ± 0.02)
queen	75 ± 25% a, a (0.23 ± 0.06)	75 ± 25% a, ab (0.17 ± 0.02)	75 ± 25% a, a (0.15 ± 0.03)

along a foraging trail. The ants killed all termites within 10–20 min after the dish was located and carried them individually back to the nest. At 48 h, 41 ± 6 % of *A. rudis* workers, 39 ± 6 % of small larvae, and 29 ± 5 % of large larvae tested positive for the marker. In comparison to the laboratory experiment where the ants fed exclusively on termites, fewer ants tested positive for the marker in the field experiment. The differences; however, were not statistically significant (*workers lab* vs. *workers field*, Kruskal-Wallis test; $\chi^2 = 0.25$, $n = 75$, $P = 0.615$; *small larvae lab* vs. *small larvae field*, Kruskal-Wallis test; $\chi^2 = 0.24$, $n = 75$, $P = 0.622$; *large larvae lab* vs. *large larvae field*, Kruskal-Wallis test; $\chi^2 = 0.75$, $n = 75$, $P = 0.387$).

Discussion

One of the goals of the laboratory feeding experiment was to determine whether predation events could be detected from predators that consumed prey that ingested the protein marker. We show that the protein marker can be readily detected in the predators for up to 72 h. Furthermore, we show that the protein marker can efficiently move through a food chain despite being subjected to digestion twice, first in the termites, and later in the ants. In previous studies that utilized protein marking to study predation, the prey were marked externally, usually by mass spraying, and were subsequently offered to the predators. One of the problems associated with protein marking and other external markers is the potential for the marker to be passed from marked prey to predators during nonpredatory events. We avoided this problem by feeding the termites IgG-treated paper which resulted in the termites retaining the marker internally. Previously, we have shown that the termites do not become externally contaminated with the marker while feeding on the IgG-treated paper, that the marker is concentrated internally, and in termite colonies is transferred to nestmates through trophallaxis and not allogrooming (Buczkowski et al., 2007). Our results demonstrate that vertebrate-specific protein markers are highly suitable to study trophic-level interactions in insects, especially in situations where the organisms are small, cryptic, highly mobile, and subterranean. Despite being subjected to digestion twice, the marker remained stable and readily detectable in ant homogenates. This indicates that the protein is stable under field conditions, resists digestion, and remains detectable despite undergoing tremendous dilution as it passes from the treated paper, through the termites, and onto the ants. As such, the marker provides a valuable tool to explore trophic interactions, particularly in studies of complex generalist predator-prey food webs.

Another objective of the laboratory feeding study was to examine the distribution of IgG-marked termite prey to the various castes and developmental stages in *A. rudis.* While numerous studies have investigated the distribution of various nutrients (e.g. sugars, proteins, and fats) in ant colonies, to our knowledge, ours is the first study to document the distribution of a complex food (i.e. whole prey) in a social insect colony. Our results show that all castes and developmental stages are fed IgG-marked fluids by trophallaxis within 24 h. The percentage of individuals testing positive increased significantly at 48 h, indicating that the intracolony distribution of termite prey continues beyond the initial 24 h. At 72 h, the percentage of individuals testing positive declined, suggesting that the marker begins to be excreted and/or metabolized after approximately 48 h. A comparison of results from the laboratory and field feeding tests revealed that fewer ant workers and larvae tested positive for the protein marker in the field. The primary reason for this difference may be larger colony size in the field and thus lower per capita ingestion of prey. Our laboratory colonies contained 200 workers and 100 brood, while field colonies of *A. rudis* may have up to 2000 workers (Headley, 1949). A second reason might be the presence of alternative food sources in the field, including alternative prey. Termite prey were the only food source available in laboratory assays. In contrast, ants in the field may have fed on various other foods including carbohydrate-rich Homopteran excretions and/or lipid-rich seeds. The effect of alternative prey and/or other food sources (e.g. carbohydrates) on the distribution of prey in colonies of *A. rudis* remains to be tested.

Investigations of the trophic ecology of forest dwelling ants and an accurate assessment of ant diets are essential in defining the role of ants in food webs. Such knowledge is especially important, given that ants and termites are a major component of tropical and temperate forest ecosystems and the most important groups in terms of biomass, number of individuals, and ecological impact (Hölldobler and Wilson, 1990). Understanding the feeding habits of predatory ants will be necessary not only to delineate the factors that affect community composition and structuring in forest ecosystems, but also to understand the proximate mechanisms that affect the evolution of sex ratios and/or reproductive allocation in ants. Larval nutrition is a critical stimulus that influences the developmental switch between workers and queens (Hölldobler and Wilson, 1990). At the colony level, workers may directly regulate sex investment ratios by selectively overfeeding certain larvae to produce either workers or gynes. Prey are an important food item for ant colonies, because they are protein-rich and may significantly influence sex investment ratios (Deslippe and Savolainem, 1995), although food supplementation experiments in forest-dwelling ants have produced mixed results (Herbers and Banschbach, 1998; Herbers and Banschbach, 1999; DeHeer et al., 2001). *A. rudis* is predominantly a scavenging ant, but it also preys on live arthropods (Southerland, 1988) and engages in myrmecochory – a mutualistic relationship with several perennial herbs, whereby the ants disperse seeds and are rewarded with a lipid-rich elaiosome (Morales and Heithaus, 1998). In *A. rudis*, colonies invested more heavily in gynes following a single provision of seeds (Morales and Heithaus, 1998). Bono and Heithaus (2002) used radio-labeled fatty acids to measure the differential uptake of elaiosome lipids among castes in *A. rudis* and found a quantitative effect of elaiosomes on larval development and gyne production. Our experiments on the distribution of IgG-marked termite prey revealed that all castes and developmental stages were fed IgG-marked fluids by trophallaxis within 24 h. Approximately 50 % of workers tested positive at 24 h and over 75 % of workers tested positive at 48 h. At any sampling time, a slightly lower percentage of larvae tested positive, although the difference between workers and larvae was often not statistically significant. The termites were most likely initially fed to the larvae, converted to liquid food, and passed to other members of the colony as is typical for all solid food entering ant colonies (Hölldobler and Wilson, 1990). It is likely that insect prey such as termites influence colony- and population-level sex ratios in *A. rudis.* However, because termite prey were distributed to all castes, it is unlikely that termites play a qualitative role in regulating gyne production. Rather, termites may have a quantitative effect on larval development, as a substantial proportion of larvae tested positive for the protein marker. Termites may be especially important in regulating sex ratios in *A. rudis* because termite colonies are stable though time and thus available as prey during the critical stage of larval development. In contrast, plant elaiosomes are only available during a narrow window of seed release, are patchily distributed, their production and quality may vary according to weather patterns, and their release may not always coincide with larval development in ant colonies (Bono and Heithaus, 2002).

Acknowledgements

We thank Joan Herbers, members of the Urban Center, and two anonymous reviewers for helpful comments on the manuscript, Kent Daane and Erik Nelson for valuable advice on immunomarking assays, and Tim Clough for statistical advice. Financial support was provided by the Industrial Affiliates Program at Purdue University.

References

Augustí N., Shayler S.P., Harwood J.D., Vaughan I.P., Sunderland K.D. and Symondson W.O.C. 2003. Collembola as alternative prey sustaining spiders in arable ecosystems: prey detection within predators using molecular markers. *Mol. Ecol.* **12**: 3467 – 3475

Breene R.G. and Sterling W.L. 1988. Quantitative phosphorus-32 labeling method for analysis of predators of the cotton fleahopper (Hemiptera: Miridae). *J. Econ. Entomol.* **81**: 1494 – 1498

Bono J.M. and Heithaus E.R. 2002. Sex ratios and the distribution of elaiosomes in colonies of the ant, *Aphaenogaster rudis. Insect. Soc.* **49**: 320 – 325

Buczkowski G. and Bennett G.W. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessile* as revealed by a protein marker. *Insect. Soc.* **53**: 282 – 290

Buczkowski G., Wang C. and Bennett G.W. 2007. Immunomarking reveals food flow and feeding relationships in the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). *Environ. Entomol.* **36**: 173 – 182

Bhatkar A.D. and Whitcomb W.H. 1970. Artificial diet for rearing various species of ants. *Florida Entomol.* **53**: 229 – 232

Carroll C.R. and Janzen D.H. 1973. Ecology of foraging by ants. *Annu. Rev. Entomol.* **4**: 231 – 257

Cornelius M.L. and Grace J.K. 1996. Effect of two ant species (Hymenopetra: Formicidae) on the foraging and survival of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Environ. Entomol.* **25**: 85 – 89

Creighton W.S. 1950. *The Ants of North America.* Bulletin of the Museum of Comparative Zoology. 585 pp

Davidson D.W. 1997. Foraging ecology and community organization in desert seed-eating ants. *Ecology* **58**: 725 – 737

DeGrandi-Hoffman G. and Hagler J.R. 2000. The flow of incoming nectar through a honey bee colony as revealed by a protein marker. *Insect. Soc.* **47**: 302 – 306

DeHeer C.J., Backus V.L. and Herbers J.M. 2001. Sociogenetic responses to ecological variation in the ant *Myrmica punctiventris* are context dependent. *Behav. Ecol. Sociobiol.* **49**: 375 – 386

Deligne J., Quennedey A. and Blum M.S. 1981. The enemies and defense mechanisms of termites. In: *Social Insects* vol. 2 (Hermann H.R., Ed), Academic Press, New York, pp 1 – 76

Deslippe R.J. and Savolainen R. 1995. Sex investment in a social insect – the proximate role of food. *Ecology* **76**: 375 – 382

Hagler J.R. 1997. Protein marking insects for mark-release-recapture studies. *Trends Entomol.* **1**: 105 – 115

Hagler J.R. 2006. Development of an immunological technique for identifying multiple predator-prey interactions in a complex arthropod assemblage. *Ann. Appl. Biol.* **149**: 153 – 165

Hagler J.R., Cohen A.C., Bradley-Dunlop D. and Enriquez F.J. 1992. Field evaluation of predation using a species- and stage-specific monoclonal antibody. *Env. Entomol.* **21**: 896 – 900

Hagler J.R. and Durand C.M. 1994. A new method for immunologically marking prey: and its use in predation studies. *Entomophaga* **39**: 257 – 265
Hagler J.R. and Jackson C.G. 2001. Methods for marking insects: current techniques and future prospects. *Annu. Rev. Entomol.* **46**: 511 – 543
Harper G.L., King R.A., Dodd C.S., Harwood J.D., Glen D.M., Bruford M.W. and Symondson W.O.C. 2005. Rapid screening of invertebrate predators for multiple prey DNA targets. *Mol. Ecol.* **14**: 819 – 828
Harwood J.D., Sunderland K.D. and Symondson W.O.C. 2004. Prey selection by linyphiid spiders: molecular tracking of the effects of alternative prey on rates of aphid consumption in the field. *Mol. Ecol.* **13**: 3549 – 3560
Headley A.E. 1949. A population study of the ant *Aphaenogaster fulva ssp. aquia* Buckley (Hymenoptera: Formicidae). *Ann. Entomol. Soc. Am.* **42**: 265 – 272
Herbers J.M. 1989. Community structure in north temperate ants: temporal and spatial variation. *Oecologia* **81**: 201 – 211
Herbers J.M. and Banschbach V.S. 1998. Food supply and reproductive allocation in forest ants: repeated experiments give different results. *Oikos* **83**: 145 – 151
Herbers J.M. and Banschbach V.S. 1999. Plasticity of social organization in a forest ant species. *Behav. Ecol. Sociobiol.* **45**: 451 – 465
Hölldobler B. and Lumsden C.J. 1980. Territorial strategies in ants. *Science* **210**: 732 – 739
Hölldobler B. and Wilson E.O. 1990. *The Ants.* The Belknap Press of Harvard University Press, Cambridge, Massachusetts. 732 pp
Ingerson-Mahar J. 2002. Relating diet and morphology in adult carabid beetles. In: *The Agroecology of Carabid Beetles* (Holland J.M., Ed), Intercept, Andover, UK, pp 111 – 136
Leponce M., Roisin Y. and Pasteels J.M. 1999. Community interaction between ants and arboreal-nesting termites in New Guinea coconut plantations. *Insect. Soc.* **46**: 126 – 130
Longhurst C., Johnson R.A. and Wood T.G. 1979. Foraging, recruitment, and predation by *Decamorium uelense* (Sanstchi) (Formicidae: Myrmicinae) on termites in Southern Guinea savanna, Nigeria. *Oecologia* **38**: 83 – 91
Lynch J.F. 1981. Seasonal, successional, and vertical segregation in a Maryland ant community. *Oikos* **37**: 183–198
McIver J.D. 1991. Dispersed central place foraging in Australian meat ants. *Insect. Soc.* **38**: 129 – 137
Mill A.E. 1983. Behavioural and toxic effects of termite defensive secretions on ants. *Physiol. Entomol.* **8**: 413–418
Morales M.A. and Heithaus E.R. 1998. Food gained from seed dispersal mutualism shifts sex ratios in colonies of the ant, *Aphaenogaster rudis. Ecology* **79**: 734–739
Nutting W.L. 1990. Insecta, Isoptera. In: *Soil Biology Guide* (Dindal D.L., Ed), Wiley and Sons, New York, pp 997 – 1032
Nienstedt K.M. and Poehling H.-M. 2004. Prey to predator transfer of enriched 1^5N-contents: basic laboratory data for predation studies using ^{15}N as marker. *Entomol. Exp. Appl*. **112**: 183–190
Oster G.F. and Wilson E.O. 1978. *Caste and Ecology in the Social Insects.* Princeton University Press, Princeton, New Jersey. 252 pp
Pasteels J.M., Deneubourg J.-L. and Goss S. 1987. Self-organization mechanisms in ant societies. (I) Trail recruitment to newly discovered food sources. In: *From Individual to Collective Behavior in Social Insects* (J.M. Pasteels and J.-L. Deneubourg, Eds). Birkhäuser, Basel, pp 43–61
SAS Institute 2002. *SAS/STAT guide for personal computers, version 8.1.* SAS Institute, Cary, NC
Sheppe W. 1970. Invertebrate predation on termites of the African savanna. *Insect. Soc.* **17**: 205 – 218
Sheppard S.K. and Harwood J.D. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. *Func. Ecol.* **19**: 751 – 762
Showler A.T., Knaus R.M. and Reagan T.E. 1988. The versatility of radiotracer methods for studying insect ethology and ecology. *Florida Entomol.* **71**: 554 – 580
Southerland M.T. 1988. The effects of temperature and food on the growth of laboratory colonies of *Aphaenogaster rudis* Emery (Hymenoptera: Formicidae). *Insect. Soc.* **35**: 305 – 309
Sunderland K.D. 1988. Quantitative methods for detecting predation occurring in the field. *Ann. Applied Biol.* **112**: 201 – 224
Symondson W.O.C. 2002. Molecular identification of prey in predator diets. *Mol. Ecol.* **11**: 627 – 641
Talbot M. 1957. Populations of ants in a Missouri woodland. *Insect. Soc.* **4**: 375 – 394
Traniello J.F.A. 1983. Social organization and foraging success in *Lasius neoniger* (Hymenoptera: Formicidae): Behavioral and ecological aspects of recruitment communication. *Oecologia* **59**: 94 – 100
Traniello J.F.A. 1989. Foraging strategies of ants. *Annu. Rev. Entomol*. **34**: 191 – 210
Wood T.G. and Sands W.A. 1978. The role of termites in ecosystems. In: *Production Ecology of Ants and Termites* (M.V. Brian, Ed), Cambridge University Press, U.K., pp 245 – 292

To access this journal online:
http://www.birkhauser.ch/IS