MOLECULAR GUT ANALYSIS OF CARABIDS (COLEOPTERA: CARABIDAE) USING APHID PRIMERS

by

Sarah Kate Wallace

A thesis submitted in partial fulfillment

of the requirements for the degree

of

Master of Science

in

Entomology

MONTANA STATE UNIVERSITY
Bozeman, Montana

August 2004

APPROVAL

of a thesis submitted by

Sarah Kate Wallace

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

Sue Blodgett

Approved for the Department of Entomology

Greg Johnson

Approved for the College of Graduate Studies

Bruce McLeod

STATEMENT OF PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a master's degree at Montana State University, I agree that the Library shall make it available to borrowers under the rules of the Library.

If I have indicated my intention to copyright this thesis by including a copyright notice age, copying is allowable only for scholarly purposes, consistent with "fair use" as prescribed in the U.S. Copyright law. Requests for permission for extended quotation from or reproduction of this thesis in whole or in parts may be granted only by the copyright holder.

Sarah Kate Wallace

30 August 04

ACKNOWLEDGEMENTS

I warmly thank Dr. Sue Blodgett and the rest of my graduate student committee for structuring my program, namely, Dr. Gregory Johnson, Dr. Kevin O'Neill, and Dr. Michael Giroux. To Brian Fitzgerald and Lynn Goosey, I extend gratitude for taking time to teach me DNA analysis laboratory methods. Patricia Denke I thank for driving the Gallatin Valley in search of experimental sites, collecting carabid beetles, and numerous other forms of entomological support and mentoring companionship. And regards to Carin Miller, for sharing her expertise on carabid identification.

I'm grateful to those who responded to letters on PCR methodology, namely Yi "Charlie" Chen, Kelly Herring, and Patricia Dodd, and finally to the landowners who allowed me to pitfall trap on their land, including the Schutters and the Milesniks.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Carabid beetles are found in a variety of Montana crops, although their impact on the food web is poorly known. To detect aphidophagy using the polymerase chain reaction (PCR), carabids were fed a single aphid and allowed to digest for increasing time intervals. Aphid-specific PCR primers were used to amplify aphid DNA from carabid beetles. For the laboratory-fed beetles, PCR detection of aphidophagy decreased with longer digestion periods. Further, there were differences among genera in the proportion of fed beetles positive for aphid DNA at the tested digestion intervals. Field surveys of 273 individual carabid beetle gut contents, representing seven genera, found all genera surveyed in Montana cropping systems, except *Microlestes* and *Pasymachus*, positive for aphid DNA. Genera positive for aphid DNA were *Calosoma, Harpalus, Pterostichus, Amara, Agonum*, and *Bembidion*. The detectability of prey DNA in the context of predator size is discussed.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

An extensive literature documents the breadth of carabid beetle larval and adult diets. Investigations of feeding habits, especially in agricultural systems, have categorized carabid food preferences as beneficial, pestiferous, or neutral, with respect to crop plants. Through observation in the laboratory and field, investigators found that most carabids are facultatively predaceous insects (Lövei and Sunderland 1996) that also scavenge dying and dead arthropods (Ball and Bousquet 2001).

The objective of this investigation is to characterize the foraging behavior of carabid genera common on Montana cropland through direct observation and to discern aphidophagous carabid genera using PCR gut analysis. To detect aphidophagy in carabid predators using PCR, currently published aphid-specific primers must be tested using laboratory-fed carabid beetles. The time frame of aphid DNA detectability, once known, may aid in estimating time of predation. Therefore, digestion trials were performed to find the half-life detectability of *Rhopalopalosipum padi* DNA amplified from DNA extracts of fed carabid beetles, using published general grain aphid primers (Chen *et al.,* 2000).

Following a brief background on carabid beetles, this thesis focuses on carabid life history and the methods employed to analyze carabid diet, including direct

observation and molecular analysis. Because of the breadth of carabid beetle diets, illustrated in the following paragraphs, it is often useful to investigate the diets of specific genera in specific locations.

Carabid Life History

The third largest family in the order Coleoptera, the Carabidae comprise approximately 40,000 described species worldwide (Lövei and Sunderland 1996). Carabid habitat ranges from caves to trees in the tropics, and from sea level to the Himilayan peaks. However, little is known of North American carabid behavior (Ball and Bousquet 2001), due to the large number of species, and their underground habitats.

Based on time of reproduction, it was once thought that carabid beetles fell into two categories: autumn and spring breeders (Larsson 1939). However, delineations of spring and autumn breeders have been challenged. Several species of carabids do not adhere to fall and spring breeding times: seven species reproduce in the winter; others reproduce twice in one year, or continuously while active. Some do not reach sexual maturity within a year, and consequently, do not mate until the following year when they are fertile (den Boer and den Boer-Daanje 1990). The original classification of spring and autumn breeders has become of limited use as the biology of many carabid species has become better known.

Of studied carabid genera, eggs are usually oviposited singly in the soil, throughout the growing season, depending on the species. Among other genera,

Pterostichus and *Calosoma* females are known to construct cells of twigs, mud, and leaf litter in which to lay their eggs.

The typical campodeiform (grub-like, flattened and elongated with well-developed legs and antennae) carabid larva burrows in the soil, pupates underground, and emerges as an adult in less than a year. Larvae may search for prey in underground burrows, or come to the surface to feed (Giglio et al. 2003). Others climb plants in search of prey. For example, adults and larvae of *Calosoma* are climbers, and prey on larvae and pupae of tree-inhabiting insects such as moths and sawflies (Ball and Bousquet 2001). Larval carabid sensory structures have been linked to prey specialization and different larval life strategies, including niche use (Giglio et al. 2003). Larval surface runners and sand diggers have more mechanoreceptors on their mouthparts than surface walkers and soil pore explorers. This may be due to the need to gauge prey size when on the run, with wide-open mandibles. In sand diggers, mouthpart mechanoreceptors may be used to detect vibrations in the substrate. Carabid larvae possess special sensitive plates for chemoreceptive sensilla on the apices of the maxillary and labial palps, optimizing chemoreception in a substrate (Sinitsyna and Chaika 2003). A larger number of labial sensilla may be related to food specialization, illustrated by *Carabus lefebvrei* Dejean, and *Licinius silphoides* Rossi, which are snail specialists. The labial sensilla may be used by these carabid larvae to detect snail trails on the substrate. Larvae which live in wetland or riverside habitats, preying at the borders of temporary waters, illustrate similar chemoreceptive equipment (Giglio et al. 2003).

As adults, carabid species are reported to live up to four years in captivity (Lövei and Sunderland 1996). However, in the field, beetles normally live for one or two seasons, and may overwinter as adults or larvae in the ground, or remain active during the winter (den Boer and den Boer-Daanje 1990). Adult beetles walk, or less commonly, fly. *Bembidion* have fully developed wings, and some can fly well, rapidly taking to the air when disturbed (Ball and Bousquet 2001).

Carabid Body Plan

Carabid beetles are physically and physiologically robust, and are one of the most successful animals in terms of diversity (Evans 1994). Primitive characters and adaptations from the basic beetle body plan include thick sclerites, protective elytra, and long, primitive 5-5-5 segmented tarsi which allow a broad stride and enable fast escapes or attacks. For defense, adult carabids also display abdominal adaptations for dispersing specialized chemical defenses. *Calosoma* give off a sweet, acrid odor when disturbed that may be related to the defensive sprays of the Bombadier beetles (Evans 1994).

Morphological adaptations of antennal structures, mouthparts, and abdominal sclerites have evolved that contribute to the success of this family. For prey or predator detection, the pro-tibial antennal cleansing organ may be used to wipe dirt or food off these chemosensory organs of the ground beetles. The antennal cleaning organ is developed, thorough and complex but slow to clean in strong ground pushers; and

primitive in fast surface runners, enabling quick cleaning. Further, specialized sensilla at the maxillary and labial palps may aid in the detection of prey or niche humidity (Giglio et al. 2003). Both adult and larval carabids possess prognathous mouthparts and enlarged, well-muscled mandibles, which aid in predation. Slight mouthpart modifications for fluid-feeding carabids include grooved mandibles with setal fringes, and the gular setal cage of Collembolan-hunting *Leistus* (Evans 1994).

Carabids display several adaptations for ground dwelling (Evans 1994). Burrowing features of the adult beetle include: a wedge-shaped body; lateral positioning of the antennae and compound eyes, conforming to the wedge-shaped body; a partial immobilization of the head, due to the cup-shaped rim of the anterior prothorax; and a strong prothoraxic box, with appropriate coxal articulation, to accommodate digging muscles for burrowing. Larvae are thought to burrow within the soil and prey upon other burrowing insects, such as cutworms and earthworms, or upon seed caches.

Carabid Habitat

Carabid habitat and microdistribution (the precise distribution of one or more kinds of organisms in a microenvironment or in part of an ecosystem) are governed by abiotic and biotic factors such as light, temperature, and humidity extremes, food supply, predator presence and distribution, and life history strategies (Thiele 1979, Lövei and Sunderland 1996).

Differences in light preferences are most marked between forest and field carabids. Most forest-dwelling and euryphotic (tolerate a wide range of light levels)

carabids prefer darkness (87% and 70%), while only 53% of field-dwelling beetles prefer darkness (Thiele 1979). *Bembidion* adults are generally day active. So are *Amara spp.,* which prefer grasslands, pastures or areas made open to light through land use practices, such as *Amara obesa* Say*,* found in sandy soils with sparse vegetation (Ball and Bousquet 2001).

Temperature is associated with carabid habitat preferences. For example, western montane *Nebria brevicollis* Fabricius distributions correlate with January isotherm mean monthly minimum temperatures along the Western coast of the United States, suggesting *N. brevicollis* seek refuge in higher altitudes which provide lower temperatures (Kavanaugh 1979). Using a thermal gradient, interspecific and intraspecific comparisons of temperature preferences using *Agonum assimile* Paykull and *Pterostichus nigrita* Paykull found interspecific temperature preferences to be statistically significant 73% of the time, while intraspecific temperature preferences were significant 17% of the time; temperature preference was species-dependent (Thiele 1979). Thiele (1979) found sixty-five percent of studied field species prefer relatively warm temperatures.

Humidity is another factor that influences the distribution of carabids. Eighty-six percent of the environmental variability in carabid habitat was explained by soil moisture and litter cover characteristics (Magagula 2003). Terrestrial carabids were classified as hygrophiles, that prefer high humidity and inhabit swamps, marshes and riparian zones; mesophiles, that prefer medium humidity and are found in damp forests and meadows; and xerophiles, that prefer low humidity, and live in dry forests, grasslands and deserts (Ball and Bousquet 2001). Among field-dwelling carabids, 59% had preferences for xeric

conditions (Thiele 1979). Examples include *Bembidion nitidum* Kirby, which are active hunters in open fields or sandy soils, and *Bembidion versicolor* LeConte, found in gravel, sand, clay, or peat. *Amara spp.* tend toward xero- or mesophilous earth-dwelling (Ball and Bousquet 2001).

Soil type was used to separate 36 carabid species into ecological groups, though the separation of assemblages was weak (Irmler 2003). Soil water content, pH, and organic content of the soils were also recorded. *Pterostichus melanarius* Illiger dominated loamy soils, while 15 of the 36 species studied by Irmler were positively correlated with the sand content of soils. Nine of the 36 species were positively correlated with soil water content (and, thus, negatively correlated with sand content, as sandy soils tend to dry rapidly), and nine were negatively correlated with soil water content. Of the nine beetle species correlated with pH, eight of them preferred lower pH soils. Only five were correlated with the organic substance of the soils (Irmler 2003).

Crop type or vegetation structure may affect carabid spatial distribution, perhaps due to the microclimatic differences found in different plant architectures, or conversely, due to the differentiation of faunal communities and faunal interactions by plant structures (Brose 2003a). Plant structures were defined by six terms: leaf type, growth type, organization of leaves, breadth in lower section, breadth in upper section, and total height. Brose found 55% of carabid species richness was explained by the heterogeneity of vegetation structures, while 18% was explained by plant species richness. This research suggests that carabids respond more to the physical structure of the environment than to

the species composition (Brose 2003a). Indeed, habitat type explained 15.7% of the variance of carabid beetle assemblages, while plant species richness explained 2.7%, and cultivated land explained another 2.7%, indicating carabid assemblages respond positively to landscape features and certain cultivated surroundings (Jeanneret et al. 2003).

Other studies associate activity densities of certain carabids with certain crop types. For example, spatial studies of 27 agricultural fields found pitfall-trapped carabid beetle *Pterostichus melanarius* most often in set-aside land and barley. *Bembidion* were abundant in oats, sugar beet, potato and set aside (Kinnunen et al. 2001), and seed generalists *Amara littoralis* Mannerheim and *Harpalus fuscipalpus* Sturm were most abundant in canola (Miller 2000). Prey preferences may also contribute to carabid habitat preferences. Evidence for prey-based carabid aggregations include findings that carabid beetles orient toward artificially created aphid patches in the field (Bryan and Wratten 1984). Factors influencing carabid activity density in rape and wheat were: density of crop stand, presence of seeds dropped on the ground, and the presence of aphids (Honek and Jarošìk 2000).

Human activity seems to affect the distribution of some carabid beetles. *Pterostichus melanarius* was accidentally introduced on both coasts of North America, spreading rapidly along ditches, road sides, cultivated fields, meadows, land around human habitations, and forests (Ball and Bousquet 2001). Because of this, it is often found in a variety of carabid studies in North America.

Land management impact on carabid biodiversity was examined in a paired-farm approach. Carabid populations were richer in species and abundance in organically

managed farms compared to Integrated Crop Management systems (no insecticides, fungicides or growth regulators, 1-2 herbicide applications), which had 36% fewer carabids (Pfiffner and Luka 2003). A decade-long study of 53 fields found no differences in indicator carabid populations between conventionally and ecologically-farmed lands, unless the farm had been ecologically run for decades, in which case carabid diversity was greater in loamy soils (Irmler 2003). Conflicting evidence that carabid diversity is associated with ecological land management practices may be due to the dispersal power of the carabids, particularly the flying species, which may explore lower-quality habitat without suffering loss in fitness costs (Brose 2003b). However, in another study, field size negatively correlated with carabid diversity, perhaps due to more refugia (headlands and field margins) in smaller fields, supporting the idea that decreasing distances from managed land to carabid habitat area results in higher carabid diversity (Irmler 2003).

Fields managed to enhance carabid activity densities, or augmented with carabid beetles, enjoy the benefit of higher rates of predation by carabids. Augmented carabid beetle activity densities correlated positively with predation rates (Landis et al. 2000). Adding filter strips increased seed predating carabid activity densities. Carabids in switchgrass filter strips aided in removing 0.6% to 4.4% of weed seed per day in soybean (Landis et al. 2000).

Carabid Foraging Behavior

Carabids forage in agreement with the enemy-free space hypothesis which states that prey will inhabit densely covered areas to avoid being seen by potential predators. Larger carabid beetles (more valuable nutritionally for predators) had higher activity densities in denser plant architectures than in open plots (Brose 2003a). The study found no evidence that vegetation structures effect the efficiency of certain hunting strategies, or that more complex vegetation supports higher carabid diversity due to more microsites for hibernation, shelter, and oviposition (Brose 2003a).

Starvation affects carabid prey choice and activity. When foraging on springtails of different sizes, female carabid *Notiophilus biguttatus* Fabricius prey choice corresponds with levels of food deprivation (Ernsting and Werf 1988). *Notiophilus biguttatus* starved for 2-3 h expend more energy catching 0.4 mg prey. However, *Notiophilus biguttatus* starved for 30 h attack large 1.0 mg prey, which decreases the ratio of energy expended over energy (food) gained, the risk being an unsuccessful attack (Ernsting and Werf 1988). In another study, starved *P. melanarius* were more active in the field than satiated beetles, possibly due to foraging activity (Fournier and Loreau 2001).

Temperature has been found to affect carabid predation activity. Increases in daily temperatures correlated with increases in slug predation by *Pterostichus madidus* Fabricius and *Harpalus rufipes* DeGeer (Ayre 2001) . At temperatures of 20^0C, *Harpalus rufipes* killed most slugs, whereas *Nebria brevicollis* Fabricus preferred to eat at 8^0C. Increased activity in

poikilothermic carabids was correlated with increasing daily field temperatures for three species of *Carabus* (Nève 1994).

Pesticide use affects carabid foraging by changing prey availability. Interestingly, *Pterostichus melanarius* had higher activity densities in chemically treated IPM fields (18-23 fungicide and insecticide treatments) than organically treated lands (10-12 treatments using S, lime, plant extracts, soap, diatom powder, or $CuSO_4$) (Labrie et al. 2003). In a separate study, the proportion of carabids *Pterostichus melanarius* and *Pterostichus madidus* which preyed upon aphids often became temporally higher after treatment with dimethon-S-methyl, because aphids fell from the plants upon insecticide treatment (Dixon and McKinley 1992). Due to scavenging on dead aphids, aphid control by carabid beetles may be overestimated in gut analysis studies not controlled for aphicide use. Further, carabid consumption of dimethoate-treated aphids led to significant mortality of *Pterostichus madidus, P. melanarius* and *Nebria brevicollis* (Dixon and McKinley 1992).

Carabid Diet

Carabid beetles generally are olfactory-tactile, polyphagous predators and scavengers, though some are parasitic (Ball and Bousquet 2001). Molluscs, ants, millipedes, and collembolans are common prey for specialized hunters. Most carabid phytophages are spermophagous.

A quarter century ago, diet information was recorded for about 150 carabid species in North America. Of these, most are opportunists, feeding on available, suitable

prey (Allen 1979). Ground beetles eat seeds (Brust and House 1988, Brust 1994, Tooley and Froud-Williams 1999, Honek and Jarošìk 2000, Langmaak et al. 2001), live and dead insects (Best and Beegle 1977, Edwards et al. 1979, Loughridge and Luff 1983, Brust et al. 1986a, b, Chiverton 1986, Hance 1987, Pollet and Desender 1987, Sunderland 1987, Hance 1990, Winder 1990, Dixon and McKinley 1992, Bilde and Toft 1994, Lys 1995, Duffield et al. 1996, Finch 1996, Johansen 1997, Chocorosqui and Paini 2000, Warner et al. 2000, Ayre 2001, Lang and Gsödl 2001), and live and dead plant and fungal tissue (Johnson and Cameron 1969, Allen 1979).

Spermophilous carabids

Field studies investigating carabid weed seed size choice found large *Harpalus caliginosis* Fabricius preferentially feeding on larger, softened seeds of wheat and sicklepod, while smaller *Harpalus pennsylvanicus* DeGeer generally consumed smaller ragweed and pigweed seeds (Brust and House 1988). Given that the 5-10% of the weed seed bank which normally germinates is within 1-2cm of soil surface, carabid beetles may access these seeds, and hence affect weed competitiveness in successive years, particularly in no-till systems (Brust and House 1988).

Predaceous or omnivorous carabids

A late 1970's literature review lists the variation in subfamily diets. For example, the subfamily Carabini are documented eating lepidopteran larvae and insects, the

Cichrini and Licinini as snail specialists, some Bembidiini as cabbage root fly egg predators, the Pterostichini, Amarini and Harpalini with varied appetites for arthropod eggs, larvae and grass, and subfamily Chalenini noted eating soft-bodied insects, annelids, mollusks, pieces of ham, and a grape (Allen 1979).

Much literature focuses on the ability of carabid beetles to decrease crop pest populations. These laboratory and field experiments generally focus on feeding studies with a few carabid species tested with homopteran, lepidopteran, and dipteran pests.

Aphid prey To study the effects of carabid aphidophagy, *Asaphidion flavipes* Linnaeus carabids were released into field cages with known sugarbeet aphid populations. *Asaphidion flavipes* controlled aphid populations at 15 individuals per $0.25m^2$, roughly natural densities (Hance 1987). *Agonum dorsal* Pont and *Pterostichus melanarius* also affected sugarbeet aphid populations, though the former only at low beetle densities, due to *P. melanarius'* tendency toward cannibalism (Hance 1987). Predator complexes which include carabids have been found more important in controlling pest populations than individual carabids. Grouped with other polyphagous predators, *Agonum dorsale, Bembidion lampros* Herbst, and *Bembidion quadrimaculatum* Say form inverse relationships with pest aphids (Edwards et al. 1979, Chiverton 1986).

Laboratory studies with four types of aphids offered to *Pterostichus cupreus* Linnaeus, *P. melanarius, P. madidus* and *Harpalus rufipes* report the Pterostichini generally eating more aphids over a 15^{o} temperature range (5^{o} to 20^{0}C) than other temperatures, and female carabids generally eating more than males (Kielty et al. 1999). *Pterostichus*

cupreus readily climbed barley plants infested with aphids when foraging alone. Interestingly, the climbing behavior was not displayed in the presence of both collembolans and aphids. The beetles exerted the least amount of energy to capture and consume low-quality prey, eating collembolans at ground level rather than climbing for aphids. Further, behavioral studies found the beetle orienting toward collembolan odor profiles, but could not conclude the same of aphid odor profiles (Mundy et al. 2000).

Lepidopteran prey *Pterostichus melanarius* was abundant in cabbage, comprising more than 50% of pitfall-trapped carabids (Shelton 1983). Other carabids have been shown to prey upon cabbage pest lepidopterans. Laboratory-fed adult *Chlaenius micans* Fabricius, *Chlaenius posticalis* Motschulsky*, Pterostichus planicollis* Motschulsky and *Dolichus halensis* Schaller averaged 21-23 4th instar diamondback moth larvae *Plutella xylostella* Linnaeus per day, while *C. micans* caused 95-99% mortality in field cages with the moth (Suenaga and Hamamura 1998). Cabbage fields surveyed for adult and larval carabids *Chlaenius micans* or *Campalita chinense* Kirby found population trends were similar to cabbage pest *Plutella xylostella and Pieris rapae* Linnaeus (Suenaga and Hamamura 1998). All stages of the voracious carabid larval stadia were found in cabbage heads year after year, implying a slight beneficial presence (Suenaga and Hamamura 1998). Carabids have also been indicated as predators of redbacked cutworm (Frank 1971).

Dipteran prey A longer beetle is assumed to have a larger gut to fill with prey. Carabid length, between 2.7 and 10 mm in a laboratory study of 35 carabid species,

related linearly to the number of cabbage root fly eggs consumed (Finch 1996). For each 1 mm increase in length up to 10 mm, the beetle could consume an extra 18 eggs (Finch 1996). Adult carabid size varies from 0.7 to 66 mm (Ball and Bousquet 2001). Evidence of beneficial predation on wheat midge larvae in spring wheat and onion fly pupae in corn further expands their contribution to dipteran pest removal (Floate et al. 1990, Menalled et al. 1999) .

Methods of Diet Analysis

Due to the diversity of carabid beetle diets, it is useful to investigate the diets of specific species in specific locations. A variety of methods used to study insect feeding preferences include observation (Sunderland 1988, Godfrey et al. 1989), gut dissection (Holopainen and Helenius 1992, Hering 1997), isotope analysis (McNabb et al. 2001), allozyme analysis (Schulz and Yurista 1995, Plague and Wallace 1998, Yi and Adams 2001), enzyme-linked immunosorbant assay (Hagler et al. 1993, McIver and Tempelis 1993, Lukasiewicz 1996, Bacher et al. 1999), and amplified DNA analysis using the polymerase chain reaction (PCR) (Asahida et al. 1997, Zaidi et al. 1999, Chen et al. 2000, Hoogendorn and Heimpel 2001, Symondson 2002).

Observation and Microscopy

With carabid beetles as objects of study, direct field observations have been uninformative and time-consuming due to nocturnal, secretive, soil-dwelling habits of

many carabids (Lys 1995). Trapping and dissecting carabid beetles for light-microscope examination of their gut contents is slightly more revealing, but time-consuming. Identifying prey parts by mounting digestive contents onto light microscope slides allowed family-level identification of Diptera, using the structure of the compound eye, antennae, legs, and wings (Pollet and Desender 1987). Similarly, sclerotized remnants of cuticle, claws, mouthparts, and legs allowed family-level identification of the larvae of flies, beetles, springtails, and other arthropods (Pollet and Desender 1987). Dissecting *Pterostichus melanarius* (about 1.6 cm long), revealed a diverse diet, including true bugs, ants, ticks, flies, beetles, and moths (Pollet and Desender 1987). Some of the carabid species dissected average 3.5 mm in length, such as *Bembidion. Bembidion* is a genus of particular interest in several studies on predation of insects including fly eggs, aphids, and springtails (Ulber et al. 1989, Wallin 1992, Humphreys and Mowat 1994, Finch 1996, Trougott 1998, Bilde et al. 2000, Honek and Jarošik 2000, Mundy et al. 2000, Suenaga and Hamamura 2001). Some *Bembidion* species feed only on body fluids, ingesting no identifiable body parts of their prey (Pollet and Desender 1987).

Serology and DNA analysis Advances in molecular technology offer additional methods for identification of indiscernible prey remnants, liquid diets, or otherwise unidentifiable prey. Molecular techniques allow researchers to survey large numbers of insects for a particular prey (and sometimes discern developmental stages within species (Hill and Crampton 1994), saving time and improving accuracy relative to gut dissections and subsequent microscopic identification of prey parts. Practical application of existing

molecular tools was previously hindered by costly, time-consuming methods. For example, protein-based insect identification requires a specific antibody developed in special tissue culture laboratories. Nevertheless, Symondson's (2002) review concluded monoclonal antibodies were the most effective method for prey detection in predator diets. Protein-based gut content analysis are well-represented in literature (Pollard 1968, Boreham and Ohiagu 1978, Lister et al. 1987, Sunderland et al. 1987, Greenstone and Morgan 1989, McIver and Tempelis 1993, Symondson and Liddell 1993, Greenstone and Trowell 1994, Hagler and Durand 1994, Hagler et al. 1994, Ayre and Port 1996, Lukasiewicz 1996, Solomon et al. 1996, Hagler and al. 1997, Hagler and Naranjo 1997, Bacher et al. 1999, Symondson et al. 1999, Symondson et al. 2000). *Trechus quadristiatus* were found to eat aphids (*Rhopalosiphum padi* Linnaeus and *Sitobion avenae* Fabricius) using ELISA, since liquid and particulate matter was all that could be seen under the microscope after dissecting 950 beetles (Sunderland et al. 1987). ELISA was used to find as little as seven ng of earthworm protein as long as 64 h after consumption by *Pterostichus melanarius* in a study investigating earthworms as alternate prey of this pest-slug predating carabid (Symondson et al. 2000). Earthworm and tipulid proteins were also consistently found, using serology, in the guts of carabid *Carabus granulatus* Duftschmidt (Lukasiewicz 1996). Several invertebrate predators, including carabid beetles, were found to have consumed aphids early in the season, at low, colonizing aphid densities, using ELISA and gut dissection (Sunderland et al. 1987).

Alternatively, prey DNA from predator gut contents can elucidate predator-prey relationships. The polymerase chain reaction (PCR) has been used by medical and

veterinary entomologists to amplify human DNA from mosquito bloodmeals (Coulson 1990, Gokool et al. 1993), host DNA from 40 yr-old alcohol-preserved haemotophagous insects (Tobolewski et al. 1992), and pathogens in gut contents of insects (Hill and Crampton 1994, Pinto et al. 2000). *Wolbachia*, a bacterial endosymbiont, was tracked by amplifying prey mite microorganisms in predatory mites (Johanowicz and Hoy 1996). Marine biologists identified stone flounder mitochondrial DNA from sand shrimp (Asahida et al. 1997). Since then, primers used for the amplification of digested insect prey DNA, using the polymerase chain reaction, have been sequenced to detect laboratory mosquito predation in Carabidae (Zaidi et al. 1999), mirid consumption of pest noctuid larvae (Agustí et al. 1999), aphidophagy by ladybird beetle larvae, lacewing larvae, a spider, and a mite (Chen et al. 2000, Cuthbertson et al. 2003, Greenstone and Shufran 2003), European corn borer predation by a coccinellid (Hoogendorn and Heimpel 2001), mosquito predation by a dragonfly (Morales et al. 2003), an pear psyllid predation by an anthocorid (Agustí et al. 2003).

However, at the time of this paper, only one published study associates a certain predator with a certain prey *in situ* using PCR gut content analysis. Investigators compared the results of PCR gut content analysis to those obtained by ELISA by running both tests to detect slug predation on each field-collected *Pterostichus melanarius* individual. Slug remains were detected for 4.3% of beetles using PCR, contrasting with the 8% positive by ELISA (Dodd *et al.*, 2003). Investigating the effect of amplicon size on PCR detection with amplicon sizes ranging from 109-294 bp did not affect slug half-life detectability in Dodd's study. However, in most studies, shorter amplicons have higher

half-life detectabilities (Agustí et al. 1999, Agustí et al. 2000, Chen et al. 2000, Hoogendorn and Heimpel 2001, Agustí et al. 2003) (Fig. 1). Further, the results of Dodd's study indicate that DNA degrades more rapidly than protein epitope in slugs, giving shorter half-lives (Dodd, *et al* 2003). Shorter half-life may aid in determining the time of a predation event without confounding successive predation events.

A review of the current literature indicates ELISA detection is affected by species (Symondson and Liddell, 1993d), sex of predator (Symondson *et al.* 1999), temperature (Sunderland, 1989), predator:prey ratio (Hagler *et al.,* 1997), quantity of target and presence of non-target protein remains in the gut (Symondson and Liddell, 1995), and

Predator	# fed prey	Amplicon	Detectability	Investigators
Carabid	2 h on Slugs	109-294	17–21 h ½ life	(Dodd et al. 2003)
Carabid	3 Mosquito larvae	146-bp 263-bp	85% at 28 h 0% after 5h	(Zaidi et al. 1999)
Ladybird	5-27 European corn borer eggs	150-bp 256-bp 369-bp 492-bp	Average 6.41 h Average 4.43 Average 4.0 Average 1.83	(Hoogendorn and Heimpel 2001)
Anthocorid	1-5 Pear psylla egg(s)	188-bp 271 bp	24.1 h ½ life 20.9 h ½ life	(Agustí et al. 2003)
Ladybird Lacewing	1 Grain aphid 1 Grain aphid	198-bp 198-bp	3.95 h ½ life 8.78 h ½ life	(Chen et al. 2000)
Spider	1 Grain aphid	198-bp	100% at 12 h	(Greenstone and Shufran 2003)
Mirid	10 Noctuid eggs	1100 600 254-bp	Not detected 0 min ½ life 45% at 4h	(Agustí et al. 1999a)
Mite	1 Apple aphid	283-bp	100% at 48 h	(Cuthbertson et al. 2003)
Dragonfly	5.5 Mosquito larvae	390-bp	Detected < 1 h	(Morales et al. 2003)

Table 1. Results of prey DNA detectability using PCR in different predator-prey systems with various amplicon sizes.

changing metabolic rates in response to starvation (Dodd, 2003). Is PCR detection of prey nucleic acid similarly affected?

Unlike ELISA, the presence of other prey in the gut does not appear to affect prey DNA detection, illustrated with congener species prey *Rhopalosiphum padi* and *Rhopalosiphum maidis* (Chen et al. 2000). *Rhopalosiphum maidis* were fed and correctly detected from ladybird larvae, lacewing larvae, and a spider, which had also eaten *Rhopalosiphum padi* (Chen et al, 2000; Greenstone and Shufran, 2003). In another study, PCR results were not affected by: the sex of a coccinellid predator (Hoogendorn and Heimpel 2001), the quantity of target DNA between 5 and 27 European corn borer eggs (Hoogendorn and Heimpel 2001), or amplicon size (Chen et al. 2000, Dodd et al. 2003).

Published data support that like ELISA results, PCR results were affected by species, illustrated with larval predator *Hippodamia convergens* and *Chrysoperla plorabunda* (Chen et al. 2000), and temperature, shown with European corn borer egg digestion by predatory adult coccinellids at either 27^0C or 20^0C (Hoogendorn and Heimpel 2001).

DNA-based techniques require a specific DNA primer, used as a probe to find the complementary prey DNA. Like developing protein prey antibodies, developing a DNA prey primer takes time, but is less costly (Chen et al. 2000, Dodd et al. 2003, Greenstone and Shufran 2003). Both protein and DNA-based techniques require specialized equipment, and respective knowledge of protein biochemistry and DNA sequencing techniques.

Currently, molecular equipment is widely available, and primer sequences for many insect predators and prey have been sequenced and are published in GenBank for

general use (Benson et al. 2004). Studies are rapidly turning to DNA-based techniques to determine predator-prey relationships (Johanowicz and Hoy 1996, Asahida et al. 1997, Agustí et al. 1999, Zaidi et al. 1999, Chen et al. 2000, Hoogendorn and Heimpel 2001, Luccini et al. 2002, Symondson 2002, Greenstone and Shufran 2003, Morales et al. 2003). Predator-prey dynamics are studied using degraded DNA, as found in animal digestive tracts and faeces, particularly for non-invasive sampling of wolves and lynx (Luccini et al. 2002, Palomeres et al. 2002). Such methods can be adapted for gut content analyses of pitfall-trapped carabid beetles.

The objective of this study was to determine the degree of aphidophagy of selected carabid beetle species in Montana dryland cropping systems, through direct observation, and PCR gut content analysis. Chapter 2 discusses molecular aphid prey detection methodology and results on laboratory-fed or field-collected predaceous carabid beetles.

CHAPTER 2

MOLECULAR GUT ANALYSIS OF CARABIDS (COLEOPTERA: CARABIDAE) USING PCR

Introduction

Carabids are well represented with descriptions for 2,625 species and subspecies in nearctic North America (Ball and Bousquet 2001). Sampling in a single Montana dryland cropping system yielded 6% of these species including tribes Notiophilini, Carabinae, Bembidiini, Pterostichini, and Harpalini, with average individual activity densities of 190.5 beetles per acre of farmland over the growing season (Miller 2000). The sheer numbers of carabids in Montana dryland cropping systems suggest their potential as pest predators (Dixon and McKinley 1992, Lys 1995, Chocorosqui and Paini 2000). A pilot study conducted in 2000 determined certain visual observations and choice tests, both in the laboratory and field, were inadequate to determine which carabid genera were aphidophagous in Montana cropping systems. Neither underground nor foliar predation events were observed, perhaps due to the accessability of a quality, vulnerable meal (Lang and Gsödl 2001). Carabids were observed scavenging upon provided dead crickets. To study aphidophagy in the field, molecular methods were used. The objective of this investigation was to discern which carabid genera eat aphids in Montana cropping

systems, using PCR gut analysis. To describe the length of aphid DNA detection after a predation event, the time at which 50% of laboratory-fed carabids tested positive for the fed aphid prey is assessed.

A progression of carabid species are active throughout the field season feeding opportunistically on varied diets, including aphids, which are widespread pests of many crops (Edwards and George 1981, Griffiths 1982). Polyphagous carabid predators are useful as biological control agents due to their early arrival in the fields, ability to curtail aphid colony growth until the arrival of stenophagous predators such as coccinellids and lacewings, and persistence of populations when aphids are absent (Sunderland et al. 1987, Holopainen and Helenius 1992). However, their beneficial impact is not well known except in a few specific cases.

Since carabid predators tend to be covert, nocturnal feeders with broad diets, techniques employed for their study include gut dissections (Pollet and Desender 1986, Dixon and McKinley 1992), laboratory observations (Griffiths 1982, Suenaga and Hamamura 1998, Kielty et al. 1999, Bilde et al. 2000, Mundy et al. 2000), field predator-prey distribution correlations (Guillemain et al. 1997), serological methods (Symondson and Liddell 1993, Ayre and Port 1996, Symondson et al. 2000, Symondson 2002, McKemey et al. 2003), or DNA analysis (Zaidi et al. 1999, Dodd et al. 2003).

Amplifying prey DNA from predator gut contents is used increasingly to elucidate predator-prey relationships, and is comparable to serology in many aspects (Symondson 2002, Dodd et al. 2003). Marine biologists identified stone flounder mitochondrial DNA from sand shrimp (Asahida et al. 1997). Primers for the

amplification of digested insect prey DNA, using PCR, have been sequenced to detect mosquito and slug predation by carabids (Zaidi et al. 1999, Dodd et al. 2003), pest noctuid larvae predation by mirids (Agustí et al. 1999), aphid predation by a spider, ladybird and lacewing larvae (Chen et al. 2000, Greenstone and Shufran 2003), European corn borer predation by a coccinellid (Hoogendorn and Heimpel 2001), mosquito predation by a dragonfly (Morales et al. 2003), and pear psylla predation by an anthicirid (Agustí et al. 2003). Only one study is published at this time which describes field surveys associating a certain predator with a specific prey (Dodd et al. 2003). Dodd found that carabid beetles consumed slugs in agricultural fields.

A review of the current literature indicates ELISA detection is affected by species (Symondson and Liddell, 1993d), sex of predator (Symondson *et al.* 1999), temperature (Sunderland, 1989), predator:prey ratio (Hagler *et al.,* 1997), quantity of target and presence of non-target protein remains in the gut (Symondson and Liddell, 1995), and changing metabolic rates in response to starvation (Dodd, 2003). Unlike ELISA, the presence of other prey in the gut does not appear to affect prey DNA detection using PCR, illustrated with congener species prey *Rhopalosiphum padi* and *Rhopalosiphum maidis* (Chen et al. 2000). Regardless of the presence of other prey, the congener species were correctly detected from ladybird larvae, lacewing larvae, and a spider (Chen et al, 2000; Greenstone and Shufran, 2003). In another study, PCR results were not affected by: the sex of a coccinellid predator (Hoogendorn and Heimpel 2001), the quantity of target DNA between 5 and 27 European corn borer eggs (Hoogendorn and Heimpel 2001), or amplicon size (Chen et al. 2000, Dodd et al. 2003). Published data support that like

ELISA results, PCR results are affected by the species of predator, illustrated using predators *Hippodamia convergens* and *Chrysoperla plorabunda* (Chen et al. 2000); and the temperature of digestion, such as European corn borer egg digestion by predatory adult coccinellids at either 27^0C or 20^0C (Hoogendorn and Heimpel 2001). The present study addresses whether PCR detection of prey nucleic acid is affected by predator:prey size ratios.

PCR primer design

As originally reported in Chen et al., 2000, aphid primers targeted for the amplification of mitochondrial COII genes resulted in quantities of aphid COII fragments that were finally sequenced. The primers amplify a 181-bp aphid mitochondrial DNA sequence, short enough to be used for amplifying degraded DNA fragments in coccinellid and lacewing larvae (Chen et al. 2000). Since there are many mitochondria per cell, amplifying mitochondrial genes generally gives more product DNA to sequence. These sequences, obtained separately from each grain aphid species, allowed the differentiation of species-specific primers for six grain aphids (Chen et al. 2000). Further, an all-aphid primer combination was recorded that would detect all grain aphids.

Chen *et al.* (2000) detected digested aphid DNA at increasing time intervals in laboratory-fed larval coccinellids and lacewings. Detectability half-lives determined for two species suggest that the DNA is digested or degraded differently in the two species. Amplifying a 198-bp fragment from aphids digested by *Chrysoperla plorabunda* Fitch gave a 3.95h half-life, while in *Hippodamia convergens,* the half-life was 8.78h. Further, amplifying

shorter fragments of DNA appears to increase half-life detectability (Chen et al. 2000, Dodd et al. 2003). For example, half-life detectability rises almost three-fold when amplifying a 198-bp fragment of aphid DNA instead of a 339-bp fragment, from *C. plorobunda.*

For the purpose of detecting an aphid meal, target DNA is extracted and amplified with PCR primers for final visualization on an agarose gel, completing the simple three-step assay: 1) extraction; 2) PCR DNA amplification; 3) band separation on agarose gel. Grain-aphid primers can accurately identify grain aphid DNA in the beetle, regardless of concomitant prey DNA, resulting in a positive or negative test (Chen et al. 2000).

The purpose of this investigation was to determine if aphid DNA could be detected in commonly trapped carabid beetles from Montana cropping systems, using PCR and grain-aphid specific primers (Chen et al. 2000). Both laboratory and field trials were employed to test the ability of PCR to detect aphidophagy.

Materials and Methods

Beetles were live-trapped in dry pitfall traps overnight. Pitfall traps were 1 L plastic soda bottles with removable cups placed inside. Bottle tops were cut and inverted to form funnels, which directed trapped insects into dry, 473 mL cups. The pitfall traps were buried with the funnel rim flush with the soil. Carabids were collected overnight twice a month, June-September. In addition, 237 mL plastic cups were placed directly in the soil for overnight carabid collection and replaced as necessary. Colonies of

Rhopalosiphum padi aphids were maintained in a growth chamber 16:8 L:D, 20^0C on potted greenhouse barley (cultivar Moravian 22).

Beetles were trapped for the feeding trials in Gallatin County, MT, in 2002 an 2003. Sites were outside of Bozeman at the Parker Farm, in grass both years; the Wright farm, in canola the first year and wheat the second; and the Milisnik ranch, in alfalfa, the second year; outside of Manhatten at the Schutter farm, in a grass strip neighboring a millet crop in 2003; and at the Department of Entomology Sustainable Pest Management crop site, located in Moore, MT, in 2002. Beetles were collected from the SPM in various crops including alfalfa, wheat, canola, barley, peas, lentils, millet, and fallow.

Laboratory

Captured adult carabid beetles were held individually in petri dishes lined with moistened filter paper in agreenhouse (Montana State University Plant Growth Center), with a photoperiod of 16:8 L:D and temperature of 20^0C, and starved for 2 to 7 days. Beetles were identified using Lindroth's keys (Lindroth 1961-69), grouped according to genera, and randomly assigned to digestion treatments.

Digestion periods were 30 (n=38), 60 (n=21), 240 (n = 37) 480 (n = 20), or 1440 (n = 7) minutes post-aphid consumption. A single aphid was offered. Beetles were observed until the aphid was eaten, allowed to digest according to treatment, and frozen in microcentrifuge tubes on dry ice for transport to the lab, where the carabids were stored at -80^0C. Carabids that did not eat the aphid were frozen as controls or removed from the experiment (n=17).

Greenhouse

Twenty-six beetles were released, individually, in terraria containing six barley plants infested with *Rhoplaosiphum padi* (bird-cherry-oat, BCO) aphids. Beetles were allowed to feed *ad libitum,* recaptured in one week and individually frozen for PCR analysis. Beetles included species from the genera *Agonum* (n=11)*, Amara* (n=4)*,* and *Pterostichus* (n=8).

Field

To detect aphidophagy in carabid genera common in Montana cropland, carabids were pitfall trapped overnight (ca. 8 hours) and frozen in microcentrifuge tubes on dry ice upon collection for transport to the laboratory. Beetles were collected monthly or biweekly June, July and August 2002, and August 2003; and from the SPM site in 2002 and the Manhattan site in 2003.

DNA extraction

Prey nucleic acid detection was performed on beetle extracts using modified methods of Chen *et al* (2000). Briefly, whole beetles were ground in 1.5 mL centrifuge tubes with a hand pestle, extracted with 500-uL extraction buffer (0.1 M $NaCl_2$, 0.2 M sucrose, 0.1 M Tris-HCl, 0.05 M EDTA and 1% SDS) and ground further. RNAse A (20 µg/mL) was added and the extract was incubated for 30 m at 65°–68°C to rid the nucleic

acid extract of RNA and denature proteins. The mixture was centrifuged for 5 m at 14,000 G.

The supernatant was cleaned of protein and lipids by phase extraction with an equal volume of phenol chloroform. DNA was precipitated by adding one-tenth vol 3M NaAc and two volumes ice-cold 95% EtOH, and incubated for one hour (0^0 C). The precipitate was centrifuged, dried, and resuspended in 200 µL dI H_2O. Template DNA extracts were stored at -80^0C and thawed at room temperature for amplification. Positive controls were 1-10 *Rhopalosiphum padi* aphids (n = 75), 1-10 95% EtOH-preserved (n=8) *Rhopalosiphum padi* aphids, and starved beetles of genera *Pterostichus, Amara, Calosoma,* and *Harpalus* ground with a single *Rhopalosiphum padi* aphid/beetle/microfuge tube (n=17). Negative controls were DNA extracts from starved beetles in the genera *Amara, Pterostichus, Microlestes* and *Harpalus* (n=15), and water (n=20). The presence of beetle DNA was not tested.

DNA amplification

Twenty-five µL polymerase chain reactions were 10 m*M* 10X buffer, pre-made by Promega (10 m*M* Tris HCl, 50 m*M* KCl, 0.1% Triton® X-100) , 7.0 m*M* $MgCl_2$, 0.1 m*M* each dNTP, 0.05 U/uL Promega *Taq* in storage buffer B (20 m*M* Tris-HCl, 100 m*M* KCl, 0.1 m*M* EDTA, 1 m*M* DTT, 50% glycerol, 1% Triton® X-100) (Promega, Madison,WI)1.0m*M* each general grain aphid primer Aphid F and Aphid R (QIAGEN Operon, Alameda, CA), and 1.0 uL template DNA from the above extraction. Aphid primer sequences are (Chen et al. 2000):

Aphid F: 5' TTTCCGATTAATTGAAGTAG 3'

Aphid R: 5' ATTCCTGGTCGGTTTATAAA 3'

Reactions were placed in 50 μL HotStart PCR tubes (Molecular BioProducts, SanDiego, CA) in two layers. The bottom layer consisted of all reagents except *Taq,* water, and template DNA, which were added just prior to thermocycling in a PTC-100 thermocycler (MJ Research, Watertown, MA, USA). DNA was denatured for 3 m at 94°C, followed by 35 amplification cycles, with 30 s denaturing at 94°C, 30 s annealing at 52° C, and 1 m extension at 72°C after amplification. Amplification products were separated on a 1.5% agarose gel submerged in 1x TBE, and visualized using ethidium bromide and *FlorS* UV scanning.

Data were analyzed using PROC GENMOD logistic regression (SAS Institute, Inc., Carey, N.C., USA), a type of regression for data with binary response variables, like PCR. Logistic data analysis is used rather than ANOVA, GLM or linear regression since, with binary data, assumptions of normality and even error distribution are violated (Allison 1999). Further, since the data was unbalanced, GENMOD was used rather than PROC LOGIT. GENMOD functions as general linear models (GLM) in logistic regression, minimizing error for unbalanced data analysis (Allison 1999).

In order to investigate if the size of beetle carcass affected PCR detection of aphid prey, beetles were divided into five classes: 1. <3mm; 2. ~7mm 3. ~10mm 4. ~15mm and 5. >20mm. Size classes included: 1. *Bembidion* and *Microlestes,* 2. *Agonum, Amara quensali,* Schonherr and *Amara littoralis* Mannerheim*,* 3. *Harpalus amputatus* Say*,*

Pterostichus scitulus LeConte, and *Pterostichus luculoblandus* Say 4. *Pterostichus melanarius* Illiger and 5. *Calosoma* and *Pasymachus.*

Results

Laboratory

The general trend of all genera suggest that aphid DNA detection decreased with time since ingestion *Amara, Pterostichus,* and *Bembidion.* Both *Amara* and *Bembidion* fed *R. padi* aphids tested positive at 8h (Fig. 1). *Pterostichus* tested positive at 4h (not tested for 8h detection due to small sample size, Fig. 1). Half-life detection was over 8 h for *Bembidion,* and less than half an hour for *Amara* and

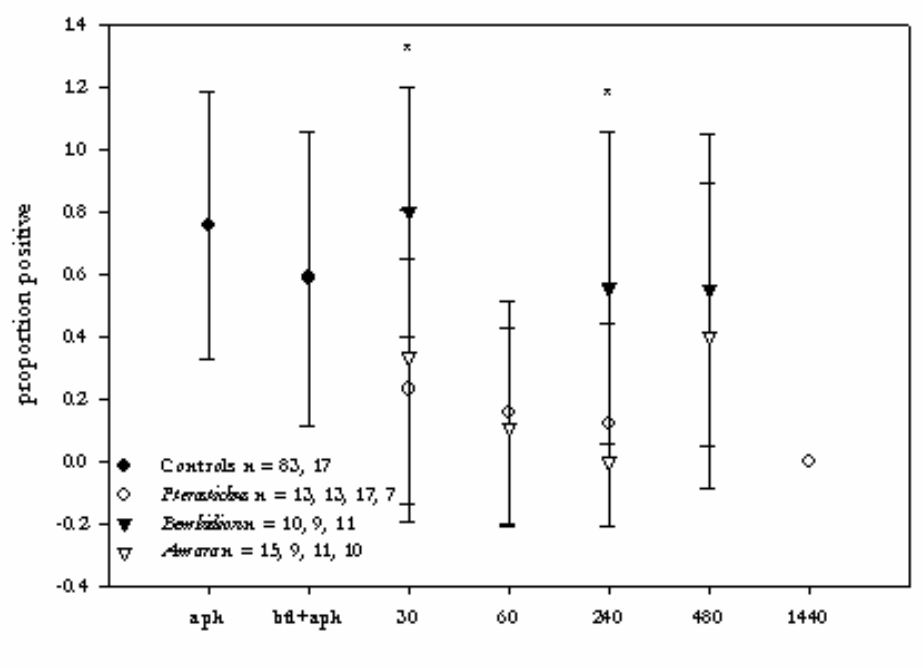


Figure 1. Proportion positive for aphid DNA detection for each genera as a function of digestion time (±SD). '*' indicates significant differences among genera in the propostion positive.

Bembidion. The proportion of *Bembidion* testing positive was higher than *Amara* or *Pterostichus* at 30 and 240 minutes (30 minutes: *Pterostichus* vs. *Bembidon* X^2 = 4.67, p = 0.036, df = 1; *Amara* vs. *Bembidion* X^2 = 6.34, p = 0.0118, df = 1. 240 minutes: *Pterostichus* vs. *Bembidon* X^2 = 4.93, p = 0.0264, df = 1; *Amara* vs. *Bembidion* X^2 = 728.64, p = 0.001, df = 1).

Grinding a *Rhopalosiphum padi* aphid with a carabid beetle carcass appeared to reduce aphid DNA detection. Aphid DNA detection was 76% (SD = 0.43), while beetle carcasses of all size classes ground with aphid DNA averaged 59% (SD = 0.47) positive. As carabid size increased from 3.0 mm to over 2.0 cm, PCR detection of the fed beetles

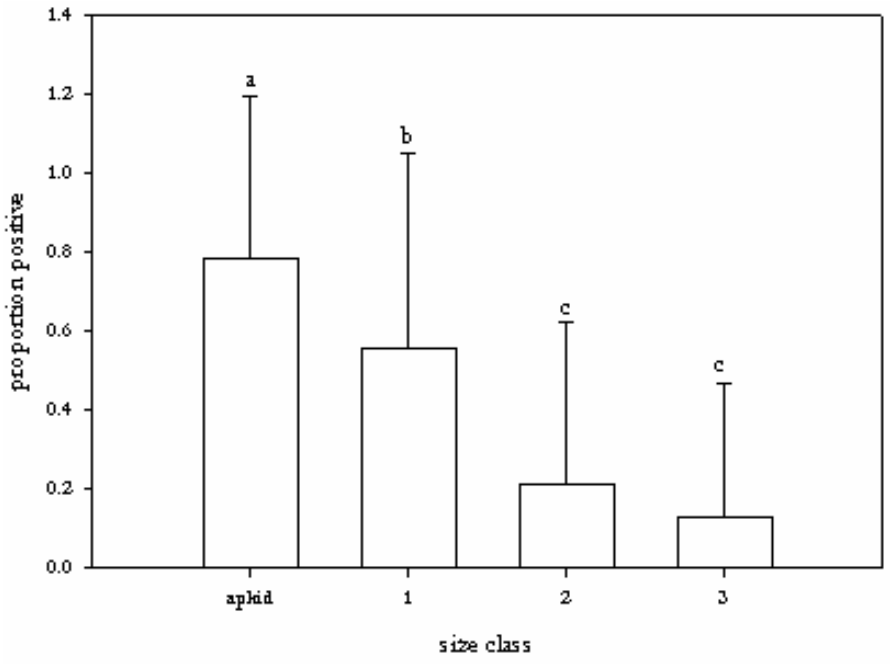


Figure 2. Proportion positive for detection of aphid DNA as a function of size classes of carabids in the laboratory (± SD). Different letters represent significant differences using PROC GENMOD at alpha = 0.05. N: aphid = 85, 1=36, 2 = 61, 3 = 61.

decreased from 78.5% to 13.1% positive (Fig. 2).

The smallest fed beetles, >3.0mm, such as *Bembidion,* tested positive for aphid DNA 2.6 times more often than ~7.0 mm beetles such as *Amara quensali* and *Agonum* spp., and 4.2 times more often than ~10mm carabids such as *Pterostichus skitulus.* There were no differences in EtOH-preserved aphid controls vs. freshly ground live aphid DNA extracts, so these positive controls were combined for analysis. DNA extracts of starved ground beetles gave no false positives.

Greenhouse

Agonum, Amara, and *Pterostichus* tested positive for aphid DNA (Fig. 3). As carabid size increased, aphid detection decreased, though the trend was not significant (Fig. 4, $p > 0.05$). However, the exact number and time of aphid ingestion was unknown.

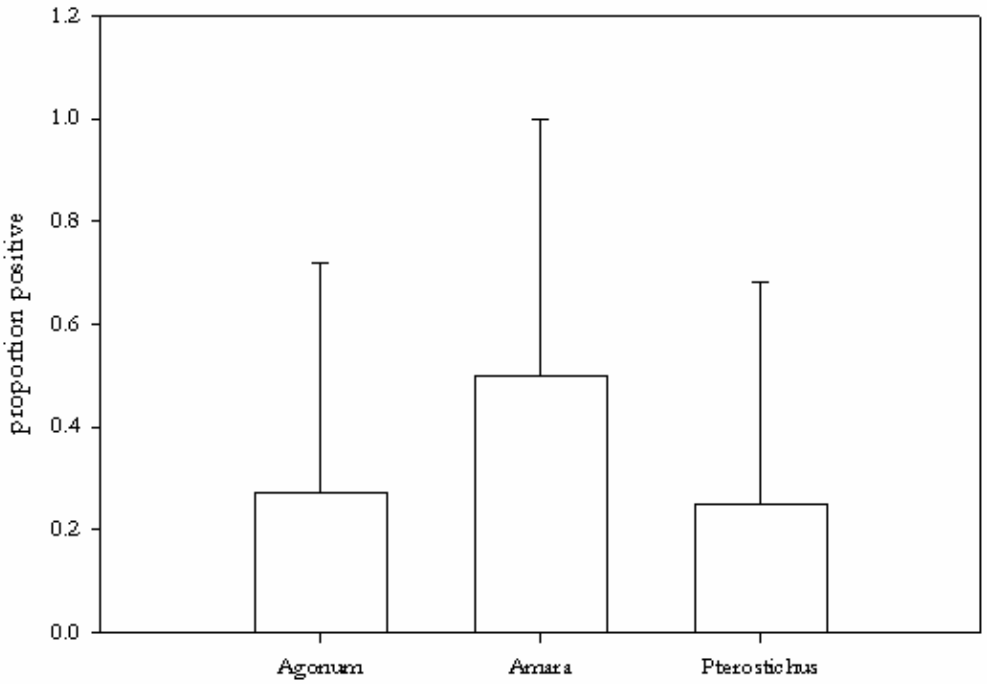


Figure 3. Proportion positive for detection of aphid DNA for carabid genera in a greenhouse study (±SD).

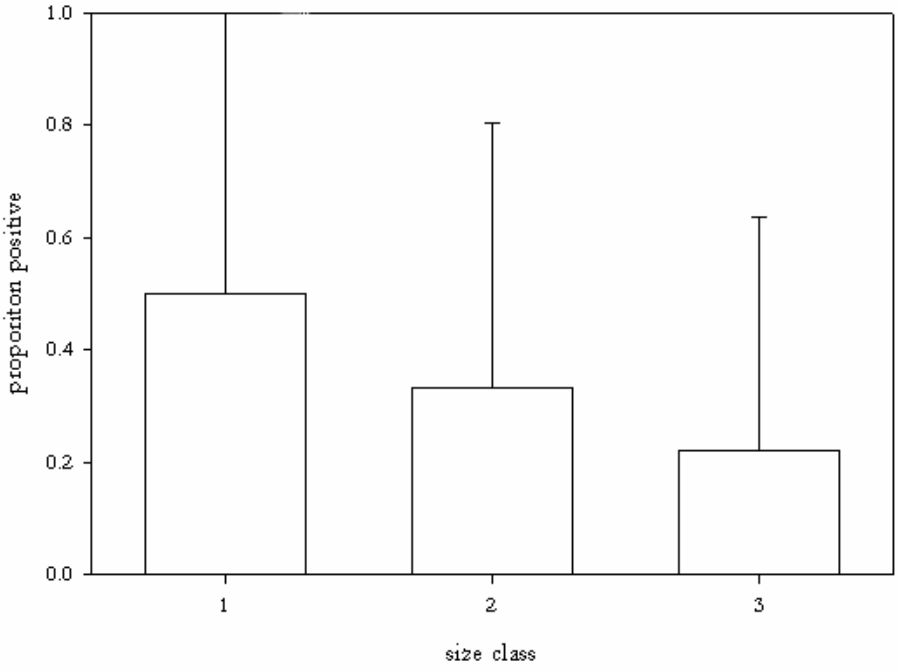


Figure 4. Proportion positive of aphid DNA detection as a function of size class of carabid beetles from a greenhouse experiment (± SD). N = 2,15,9

Field

All tested field genera were positive for aphid DNA with the exception of *Microlestes* (N=2) and *Pasymachus* (N= 2) (Fig. 5). The general trend of decreasing PCR detection with increasing size held in the field (Fig. 6, $p > 0.05$), and may be affected by the time (unknown) last ingestion of an aphid meal. Of the beetles collected in 2002, (N = 125)

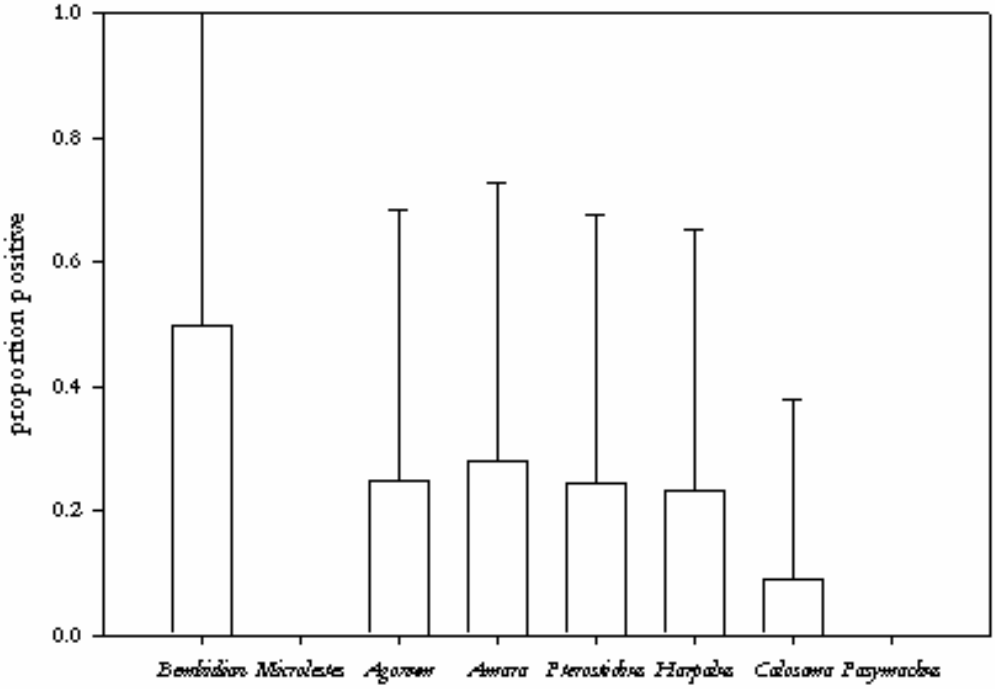


Figure 5. Propostion positive of aphid DNA as a function of carabid genus from field collections (+ SD). N = 2, 2, 12, 29, 65, 52, 22, 2.

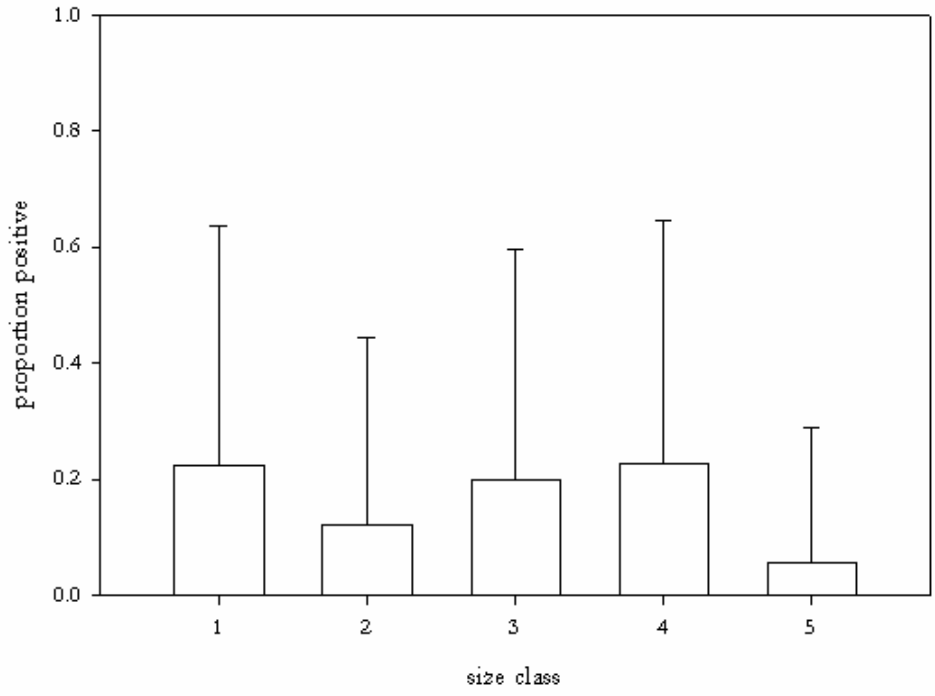


Figure 6. Proportion positive of aphid DNA as a function of size classes of carabid beetles from field collections (+SD). N: 1 = 9, 2 = 92, 3 = 111, 4 = 22, 5 = 35.

only July individuals tested positive for aphid DNA, at 16.5%. In 2003, 39.3% of collected beetles tested positive for aphid DNA in August (N = 56). Both the SPM and the Gallatin Valley sites yielded beetles of which 21.9% were positive for aphids (N =183). Figure 7 shows the 181-bp products of the PCR reaction using grain aphid primers.

.

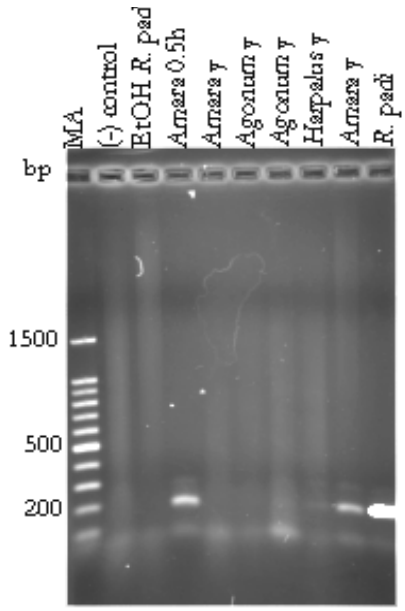


Figure 7. Amplification products using grain aphid primers on a 1.5% agarose gel. *R. padi,* (-) control and ETOH *R. padi* (positive and two negative controls). *Amara* 0.5 h is an *Amara* beetle at 30 minute digestion. 'y' indicates genera that were field collected.

Discussion

PCR analysis

Field surveys of predator-prey relationships can be confirmed using DNA gut content analysis. Indeed, of 273 individual carabids representing seven genera, 21.9% of all tested carabids were positive for aphid DNA when analyzed directly from the field. Though the laboratory half-lives for aphid DNA detection were short in *Pterostichus* and *Amara,* the field data suggest PCR is still useful for detecting predator-prey relationships in these genera. Six of the eight genera tested positive as predators of aphids in cropland.

The laboratory results support the idea that gut content analysis of field-collected carabid beetles using PCR is feasible, as long as the pitfall-trapped carabids are preserved upon collection or analyzed immediately. The ability to detect a single aphid's digested DNA in *Amara* and *Bembidion* for over 8 hours, and *Pterostichus* for over 4 hours (Fig. 1), is superior to prey detection in dragonflies of less than one hour (Morales et al. 2003), comparable to mirids tested after 4 hours (45% detection) (Agustí et al. 1999) and ladybird beetle prey detection of 1.83 – 6.41 h (Hoogendorn and Heimpel 2001). PCR has been successfully used in pitfall trapped *Pterostichus melanarius* to test for slug remains (Dodd et al. 2003).

Current investigations comparing prey DNA detection to ELISA found both tests are affected by factors that affect digestion (Morales et al. 2003). ELISA detection is affected by species (Symondson and Liddell, 1993d), sex of predator (Symondson *et al.* 1999), temperature (Sunderland, 1989), predator:prey ratio (Hagler *et al.,* 1997), quantity of target and presence of non-target protein remains in the gut (Symondson and Liddell, 1995), and changing metabolic rates in response to starvation (Dodd, 2003).

Unlike ELISA, prey DNA detection is not affected by the presence of other prey in the gut, the sex of a coccinellid predator (Hoogendorn and Heimpel 2001), or the quantity of target DNA between 5 and 27 European corn borer eggs (Hoogendorn and Heimpel 2001). Like ELISA results, PCR results are affected by the species of predator (Chen et al. 2000), temperature of prey digestion, and amplicon size (Hoogendorn and Heimpel 2001), though there is evidence suggesting no effect of amplicon size (Chen et al. 2000, Dodd et al. 2003).

The laboratory, greenhouse, and field results here indicate that predator:prey size ratios affect prey detection using PCR (Figs 2, 4, 6). *Bembidion,* one of the smallest of the carabids examined hardly doubles the size of its larger aphid prey, tested positive more often than larger carabid genera. Similar effects of predator: prey size ratio on prey detectability were found using ELISA, which was more effective at detecting pink bollworm egg antigens in smaller minute pirate bug *Orius insidius* Say predators than larger ladybug *Hippodamia convergens* predators (Hagler and al. 1997, Hagler and Naranjo 1997). In the field, size class predictions were weakly supported, even though detection time was limited and the last aphid ingestion event is unknown. Further, it is not known whether a predator of an aphidophagous prey species would test positive for the prey's aphid meal. Though food-chain affects were not found in serological gut analysis (Harwood et al. 2001), predation by larger beetles on prey which have eaten aphids may confound aphid DNA analysis results.

Except *Bembidion* species, adult carabid beetles had lower aphid DNA detectability than did larval lacewings and ladybugs fed one *Rhopalosiphum maidis* aphid, perhaps due to size. Starved carabids ground with an added aphid averaged a 17% lower aphid DNA detection than aphids ground alone, indicating the beetle carcass interfered with aphid DNA detection, using PCR (Fig. 1).

Dissecting the gut is simple and may circumvent size constraints, though dissection does not appear to aid PCR detection. Dragonfly larva fore-, mid-, and hindguts were removed and separately analyzed for mosquito larvae using PCR, but no

increase in amplification products was found (Morales et al. 2003). Such dissections further allow visual examination of the gut contents, and, if the prey is identifiable, may be adequate to describe prey without DNA methodology. Morales (2003) visually confirmed that mosquito larvae were absent from the fed dragonflies using dissection.

The laboratory results further support that species affect prey DNA half-life detection. A single aphid digested by *Bembidion* had longer half-life detection than *Amara* or *Pterostichus* (Fig. 1). The half-life of aphid DNA in *Bembidion* of over 8 h was comparable to that found for predators *Hippodamia convergens* and *Chrysoperla plorabunda. Hippodamia convergens* and *Chrysoperla plorabunda* had detectability half-lives of 8.78 h and 3.95 h, respectively. Thus, a positive result is 2.2 times more likely with a *H. convergens* individual than a *C. plorabunda* individual in laboratory-fed beetles, indicating the relative "weight" of a positive test in different predators (Chen et al. 2000).

The effects of temperature on digestion, and hence PCR prey detection, are unknown in the different genera studied. Increasing field temperatures have a greater impact on insects with a smaller surface area to volume ratio, possibly causing smaller insects to digest more rapidly. Subsequent decreases in prey protein antigen detection with increasing temperature were found using ELISA (Giller 1984).

In the present study, because every possible prey type in the field was not subject to amplification using the grain aphid primers, the results must be viewed critically. The general grain aphid primer was found to amplify aphid DNA from non-grain aphids, including *Myzus persicae* Sulzer (data not shown). Pea and canola aphids were the most

commonly collected aphids at the SPM site (M. Rolston, pers. comm.) Future surveys should record the prey complex and test a few of the available prey items for false positives using their specific PCR primers. For example, representative members of nine families were tested to illustrate the usefulness of pear psylla primers for trophic studies, resulting in false positives for the pear psyllid only within the Psyllidae (Agustí et al. 2003).

To detect DNA concentration effects, further studies would necessarily include estimates of template DNA concentrations. To further address the relationship of predator:prey mass ratio on aphid detection using PCR, the exact predator:prey mass ratio might be recorded. In the current investigation, the ratio is roughly estimated here by carabid length. Alternately, dilutions of DNA extracts have been found to increase PCR detection (Chen et al. 2000). Pilot dilution series were tried with the carabid beetle DNA extracts but yielded similar results to those using undiluted beetle DNA extracts.

In conclusion, I found that carabids in Montana eat aphids, which can be tested using PCR and aphid primers. Further, the data suggest that the window of opportunity for detecting prey aphid DNA differs with genus, as well as time since ingestion, and predator:prey size ratio. Future studies might address the effect of temperature and food chain effects on aphid detection using PCR. Such studies will become increasingly important as more field surveys are conducted to confirm predator-prey relationships.

LITERATURE CITED

Agustí, N., T. Unruh, and S. Welter. 2003. Detecting *Cacospsylla pyricola* (Hemiptera:Psyllidae) in predator guts using CIII mitochondrial markers. Bulletin of Entomological Research **93**:179-185.

Agustí, N., M. C. D. Vicente, and R. Gabarra. 1999. Development of sequence amplified characterized region (SCAR) markers of *Helicoverpa armigera*: a new polymerase chain reaction-based technique for predator gut analysis. Molecular Ecology **8**:1467-1474.

Agustí, N., M. D. Vincete, and R. Gabarra. 2000. Developing SCAR markers to study predation on *Yrialeurides vaporariorum*. Insect Molecular Biology.

Allen, R. T. 1979. The occurrence and importance of ground beetles in agricultural and surrounding habitats. *in* T. L. Erwin, G. E. Ball, and D. R. Whitehead, editors. Carabid beetles: Their evolution, natural history, and classification. W. Junk, The Hague.

Allison, P. D. 1999. Logistic Regression Using the SAS System: Theory and Application. SAS Institute, Inc, Cary, N.C.

Asahida, T., Y. Yamashita, and T. Kobayashi. 1997. Identification of consumed stone flounder, *Kareius bicoloratus* (Basilewsky), from the stomach contents of sand shrimp, *Crangon affinis* (De Haan) using mitochondrial DNA analysis. Journal of Experimental Marine Biology and Ecology **217**:153-163.

Ayre, K. 2001. Effect of predator size and temperature on the predation of *Deroceras reticulum* (Muller) (Mollesca) by carabid beetles. Journal of Applied Entomology **125**:389-395.

Ayre, K., and G. R. Port. 1996. Carabid beetles recorded feeding on slugs in arable field using ELISA. Pages 411-418 *in* 1996 BCPC Symposium Proceedings.

Bacher, S., D. Schenk, and H. Imboden. 1999. A monoclonal antibody to the shield beetle *Cassida rubiginosa* (Coleoptera, Chrysomelidae): A tool for predator gut analysis. Biological Control **16**:299-309.

Ball, G. E., and Y. Bousquet. 2001. CARABIDAE Latreille. Pages 32-132 *in* R. Arnett and M. Thomas, editors. American Beetles. CRC Press, Boca Raton, FL, USA.

Benson, D. A., I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and D. L. Wheeler. 2004. GenBank: update. Nucleic. Acids Research **32**:D23-26.

Best, R. L., and C. C. Beegle. 1977. Food preferences of five species of carabids commonly found in Iowa cornfields. Environmental Entomology **6**:9-12.

Bilde, T., J. Axelsen, and S. Toft. 2000. The value of Collembola from agricultural soils as food for a generalist predator. Journal of Applied Ecology **37**:672-683.

Bilde, T., and S. Toft. 1994. Prey preference and egg production of the cereal carabid beetle *Agonum dorsale*. Entomolgia Experimentalis et Applicata **73**:151-156.

Boreham, P. F. L., and C. E. Ohiagu. 1978. The use of serology in evaluating invertebrate predator-prey relationships: a review. Bulletin of Entomological Research **68**:171-194.

Brose, U. 2003a. Bottom-up control of carabid beetle communities in early successional wetlands: mediated by vegetation structure or plant diversity? Oecologia **135**:407-413.

Brose, U. 2003b. Island biogeography of temporary wetland carabid beetle communities. Journal of Biogeography **30**:879-888.

Brust, G. E. 1994. Seed-predators reduce broad leaf weed growth and competitive ability. Agriculture, Ecosystems and Environment **48**:27-34.

Brust, G. E., and G. J. House. 1988. Weed seed destruction by arthropods and rodents in low-input soybean agroecosystems. American Journal of Alternative Agriculture **3**:19-25.

Brust, G. E., B. R. Stinner, and D. A. McCartney. 1986a. Predation by soil inhabiting arthropods in intercropped and monoculture agroecosystems. Agriculture, Ecosystems, and Environment **18**:145-154.

Brust, G. E., B. R. Stinner, and D. A. McCartney. 1986b. Predator activity and predation in corn agroecosystems. Environmental Entomology **15**:1017-1021.

Bryan, K. M., and S. D. Wratten. 1984. The responses of polyphagous predators to prey spatial heterogeneity:aggregation by carabid and staphylinid beetles to their cereal aphid prey. Ecological Entomology **9**:251-259.

Chen, Y., K. L. Giles, M. E. Payton, and M. H. Greenstone. 2000. Identifying key cereal aphid predators by molecular gut analysis. Molecular Ecology **9**:1887-1898.

Chiverton, P. A. 1986. Predator density manipulation and its effects on populations of *Rhopalosiphum padi* (Hom.: Aphididae) in spring barley. Annals of Applied Biology **109**:49-60.

Chocorosqui, V. R., and A. Paini. 2000. Predation of *Alabama argillacea* (Hübner) (Lepidoptera: Noctuidae) pupae by larvae and adults of *Calosoma granulatumI* Perty (Coleoptera: Carabidae) in the laboratory. Anais de Sociodad Entomologica do Brasil **29**:65-70.

Coulson, R. M. R. 1990. Amplification and analysis of human DNA present in mosquito bloodmeals. Medical and Veterinary Entomology **4**:357-366.

Cuthbertson, A. G. S., C. C. Fleming, and A. K. Murchie. 2003. Detection of *Rhopalosiphum insertum* (apple-grass aphid) predation by the predatory mite *Anystis baccarum* using molecular gut analysis. Agricultural and Forest Entomology **5**:219-225.

den Boer, P. J., and W. den Boer-Daanje. 1990. On life history tactics in carabid beetles: are there only spring and autumn breeders? Pages 247-258 *in* N. E. Stork, editor. The Role of Ground Beetles in Ecological and Environmental Studies. Intercept, Ltd., Andover, Hampshire.

Dixon, P. I., and R. G. McKinley. 1992. Pitfall trap catches of and aphid predation by *Pterostichus melanarius* and *Pterostichus madidus* in insecticide treated and untreated potatoes. Entomologia Experimentalis et Applicata **64**:63 - 72.

Dodd, C., M. Bruford, and W. Symondson. 2003. Detection of slug DNA within carabid predators using prey-specific PCR primers. Pages 13-20 *in* BCPC symposium proceedings, no. 80. Slugs and Snails: Agricultural, Veterinary and Environmental Perspectives, University College, Canterbury, Kent.

Duffield, S. J., P. C. Jepson, S. D. Wratten, and N. W. Sotherton. 1996. Spatial changes on invertebrate predation rate in winter wheat following treatment with dimethoate. Entomologia Experimentalis et Applicata **78**:9-17.

Edwards, C. A., and K. S. George. 1981. Carabids as predators of cereal aphids. Pages 191-199 *in* British Crop Protection Conference - Pests and Diseases. The British Crop Protection Council, Lavenhayn Press Ltd., Brighton, England.

Edwards, C. A., K. D. Sunderland, and K. S. George. 1979. Studies on polyphagous predators of cereal aphids. Journal of Applied Ecology **16**:811-823.

Ernsting, G., and D. C. Van der Werf. 1988. Hunger, partial consumption of prey and prey size preference in a carabid beetle. Ecological Entomology:155-164.

Evans, M. E.G. 1994. The carabid body plan: a functional interpretation. Pages 25-31 *in* K. Desender, M. Dufrene, M. Loreau, M. L. Luff, and J.-P. Maelfait, editors. Carabid Beetles: Ecology and Evolution. Kluwer Academic Publishers, Dordrecht.

Finch, S. 1996. Effect of beetle size on predation of cabbage root fly eggs by ground beetles. Entomologia Experimentalis et Applicata **81**:199-206.

Floate, K. D., J. F. Doane, and C. Gillott. 1990. Carabid predators of the wheat midge (Diptera: Cecidomyiidae) in Saskatchewan. Environmental Entomology **19**:1503-1511.

Fournier, E., and M. Loreau. 2001. Activity and satiation state in *Pterostichus melanarius:* an experiment in different agricultural habitats. Ecological Entomology **26**:235-244.

Frank, J. H. 1971. Carabidae (Coleoptera) as predators of the redbacked cutworm (Lepidoptera: Noctuidae) in central Alberta. Canadian Entomologist **103**:1039-1044.

Giglio, A., E. A. Ferrero, E. Perrptta, S. Tripepi, and T. Z. Brandmayr. 2003. Ultrastructure and comparative morphology of mouth-part sensilla in ground beetle larvae (Insecta, Coleoptera, Carabidae). Zoologischer Anzieger **2442**:277-292.

Giller, P. S. 1984. Predator gut state and prey detectability using electrophoretic analysis of gut contents. Ecological Entomology **9**:157-162.

Godfrey, K. E., W. H. Whitcomb, and J. L. Stimac. 1989. Arthropod predators of velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Noctuidae), eggs and larvae. *Environmental Entomology* **18**:118-123.

Gokool, S., C. Curtis, and D. F. Smith. 1993. Analysis of mosquito bloodmeals by DNA profiling. Medical and Veterinary Entomology **7**.

Greenstone, M. H., and C. E. Morgan. 1989. Predation on *Heliothis zea* (Lepidoptera: Noctuidae): an instar-specific ELISA for stomach analysis. Annals of the Entomological Society of America **82**:45-49.

Greenstone, M. H., and K. A. Shufran. 2003. Spider predation: species-specific identification of gut contents by polymerase chain reaction. The Journal of Arachnology **31**:131-134.

Greenstone, M. H., and S. C. Trowell. 1994. Arthropod predation: a simplified immudot format for predator gut analysis. Annals of the Entomological Society of America **87**:214-217.

Griffiths, E. 1982. The carabid *Agonum dorsale* as a predator in cereals. Annals of Applied Biology **101**:152-154.

Guillemain, M., M. Loreau, and T. Daufresne. 1997. Relationships between the regional distribution of carabid beetles (Coleoptera: Carabidae) and the abundance of their potential prey. Acta Oecologia **18**:465-483.

Hagler, J. R., and e. al. 1997. Immunological examinations of species variability on predator gut content assays: Effect of predator:prey protein ratio in immunoassay sensitivity. Biological Control **9**:120-128.

Hagler, J. R., A. G. Brower, Z. Tu, D. N. Byrn, D. Bradley-Dunlop, and F. J. Enriquez. 1993. Development of a monoclonal antibody to detect predation of the sweet potato whitefly, *Bemisia tabaci*. Entomologia Experimentalis et Applicata **68**:231-236.

Hagler, J. R., and C. M. Durand. 1994. A new method for immunologically marking prey and its use in predation studies. Entomophaga **39**:257-265.

Hagler, J. R., and S. E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: detectability of prey remains in relation to predator species, temperature, time, and meal size. Biological Control **9**:112-119.

Hagler, J. R., S. E. Naranjo, D. Bradley-Dunlop, F. J. Enriquez, and T. J. Henneberry. 1994. A monoclonal antibody to pink bollworm (Lepidoptera: Gelechiidae) egg antigen: A tool for predator gut analysis. Ecology and Population Biology **87**:085-090.

Hance, T. 1987. Predation impact of carabids at different population densities on *Aphis fabae* development in sugar beet. Pedobiologia **30**:251-262.

Hance, T. 1990. Relationships between crop types, carabid phenology, and aphid predation in agroecosystems. Pages 55-63 *in* N. E. Stork, editor. The Role of Ground Beetles in Ecological and Environmental Studies. Intercept, Ltd., Andover, Hampshire.

Harwood, J. D., S. W. Phillips, K. D. Sunderland, and W. O. C. Symondson. 2001. Secondary predation: quantification of food chain errors in an aphid-spider-carabid system using monoclonal antibodies. Molecular Ecology **10**:2049-2057.

Hering, D. 1997. Riparian ground beetles preying on aquatic invertebrates: a feeding strategy in alpine floodplains. Oecologia **111**:261-270.

Hill, S. M., and J. M. Crampton. 1994. DNA-based methods for the identification of insect vectors. Annals of Tropical Medicine and Parasitology **88**:227-250.

Holopainen, J. K., and J. Helenius. 1992. Gut contents of ground beetles (Col., Carabidae), and activity of these and other epigeal predators during an outbreak of *Rhopalosiphum padi* (Hom., Aphididae). Acta Agriculturae Scandinavica, Section B, Soil and Plant Science **42**:56-61.

Honek, A., and V. Jarošik. 2000. The role of crop density, seed and aphid presence in diversification of field communities of Carabidae (Coleoptera). Environmental Journal of Entomology **97**:517-525.

Hoogendorn, M., and G. E. Heimpel. 2001. PCR-based gut content analysis of insect predators: using ribosomal ITS-1 fragments from prey to estimate predation frequency. Molecular Ecology **10**:2059-2067.

Humphreys, I. C., and D. J. Mowat. 1994. Effects of some organic treatments on predators (Coleoptera: Carbidae) of cabbage root fly, *Delia radicum* (L.) (Diptera: Anthomyiidae), and on alternative prey species. Pedobiologia **38**:513-518.

Irmler, U. 2003. The spatial and temporal pattern of carabid beetles on arable fields in northern Germany (Schleswig-Holstein) and their value as ecological indicators. Agriculture, Ecosystems, and Environment **98**:141-151.

Jeanneret, P., B. Schu:pbach, and H. Luka. 2003. Quantifying the impact of landscape and habitat features on biodiversity in cultivated landscapes. Agriculture, Ecosystems, and Environment **98**:311-320.

Johanowicz, D. L., and M. A. Hoy. 1996. *Wolbachia* in a predator-prey system: 16S ribosomal DNA analysis of two phytoseiids (Acari: Phytoseiidae) and their prey (Acari: Tetranychidae). Annals of the Entomological Society of America **89**:435-441.

Johansen, N. S. 1997. Mortality of eggs, larvae and pupae and larval dispersal of the cabbage moth, *Mamestra brassicae,* in white cabbage in south-eastern Norway. Entomologia Experimentalis et Applicata **83**:347-360.

Johnson, N. E., and R. S. Cameron. 1969. Phytophagous ground beetles. Annals of the Entomological Society of America **62**:909-914.

Kavanaugh, D. H. 1979. Investigations on present climactic refugia in North America though studies on the distributions of carabid beetles: concepts, methodology and prospectus. *in* T. L. Erwin, G. E. Ball, and D. R. Whitehead, editors. Carabid Beetles: Their Evolution, Natural History, and Classification, Boston.

Kielty, J. P., L. J. Allen-Williams, and N. Underwood. 1999. Prey preferences of six species of Carabidae (Coleoptera) and one Lycosidae (Araneae) commonly found in UK arable crop fields. Zeitschrift fur angewandte Entomologie **123**:193.

Kinnunen, H., J. Tiainen, and H. Tukia. 2001. Farmland carabid beetle communities at multiple levels of spatial scale. Ecography **24**:189-197.

Labrie, G., C. Prince, and J.M. Bergeron. 2003. Abundance and developmental stability of *Pterostichus melanarius* (Coleoptera: Carabidae) in organic and integrated pest management orchards of Québec, Canada. Entomologicheskae-Obozrenie:123-132.

Landis, D. A., F. D. Menalled, J. C. Lee, D. M. Carmona, and A. Péréz-Valdéz. 2000. Habitat Management to Enhance Biological Control in IPM. Pages 226-239 *in* Emerging technologies for integrated pest management: concepts, research and implementation. APS Press, St. Paul, Minn.

Lang, A., and S. Gsödl. 2001. Prey vulnerability and active predator choice as determinants of prey selection: a carabid beetle and its aphid prey. Journal of Applied Entomology **125**:53-61.

Langmaak, M., S. Land, and W. B. Fix. 2001. Effects of different field management systems on the carabid coenosis in oil seed rape with special respect to ecology and nutritional status of predacious *Poecilus cupreus* L. (Col., Carabidae). Journal of Applied Entomology **125**:313-320.

Larsson, S. G. 1939. Entwicklungstypen und Entwicklungszeiten der danischen Carabiden. Entomologiske Meddedelelser **20**:277-560.

Lindroth, C. H. 1961-69. The ground beetles (Carabidae, exc. Cicindelinae) of Canada and Alaska Parts 1-6. Opuscula Entomologica **xlvii**:1192 pp.

Lister, A., M. B. Usher, and W. Block. 1987. Description and quantification of field attack rates by predatory mites: an example using an electrophoresis method with a species of Antarctic mite. Oecologia **72**:185-191.

Loughridge, A. H., and M. L. Luff. 1983. Aphid predation by *Harpalus rufipes* (DeGeer) (Coleoptera: Carabidae) in the laboratory and field. Journal of Applied Ecology **20**:451-462.

Lövei, G. L., and K. D. Sunderland. 1996. Ecology and behavior of ground beetles (Coleoptera: Carabidae). Annual Review of Entomology **41**:231-256.

Luccini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Molecular Ecology **11**:857-868.

Lukasiewicz, J. 1996. Predation by the beetle *Carabus granulus* L. (Coleoptera, Carabidae) on soil microfauna in grassland on drained peats. Pedobiologia **40**:364-376.

Lys, J.A. 1995. Observation of the epigeic predators and predation on artificial prey in a cereal field. *Entomologica Experimentalis et Applicata* **75**:265-272.

Magagula, C. N. 2003. Changes in carabid beetle diversity within a fragmented agricultural landscape. African Journal of Ecology **41**:23-30.

McIver, J. D., and C. H. Tempelis. 1993. The arthropod predators of ant-mimetic and aposematic prey: a serological analysis. Ecological Entomology **18**:218-222.

McKemey, A., W. Symondson, and D. Glen. 2003. Predation and prey size choice by the carabid beetle *Pterostichus melanarius* (Coleoptera: Carabidae): the dangers of extrapolating from laboratory to field. Bulletin of Entomological Research **93**:227-234.

McNabb, D. M., J. Halaj, and D. H. Wise. 2001. Inferring trophic positions of generalist predators and their linkage to the detrital food web in agroecosystems: a stable isotope analysis. Pedobiologia **45**:289-297.

Menalled, F. D., J. C. Lee, and D. A. Landis. 1999. Manipulating carabid beetle abundance alters prey removal rates in corn fields. BioControl **43**:441-456.

Miller, C. 2000. Carabid beetle (Coleoptera: Carabidae) seasonal occurrence and species composition in northern Montana cropping systems. M.S. Montana State University, Bozeman, MT.

Morales, M. E., D. M. Wesson, I. W. Sutherland, D. E. Impoinvil, C. M. Mbogo, J. I. Githure, and J. C. Beier. 2003. Determination of *Anopholes gamibiae* larval DNA in the gut of insectivorous dragonfly (Libellulidae) nymphs by polymerase chain reaction. Journal of the American Mosquito Control Association **19**:163-165.

Mundy, C. A., L. J. A. Williams, N. Underwood, and S. Warrington. 2000. Prey selection and foraging behaviour by *Pterostichus cupreus* L. (Col., Carabidae) under laboratory conditions. Journal of Applied Entomology **124**:349 - 358.

Nève, G. 1994. Influence of temperature and humidity on activity of three *Carabus* species. Pages 189-192 *in* K. Desender, M. Dufrene, M. Loreau, M. L. Luff, and J.-P. Maelfait, editors. Carabid Beetles: Ecology and Evolution. Kluwer Academic Publishers, Dordrecht.

Palomeres, F., J. Godoy, A. Piriz, S. O'Brian, and W. Johnson. 2002. Faecal genetic analysis to determine the presence and distribution of elusive carnivores: design and feasibility for the Iberian lynx. Molecular Ecology **11**:2171-2182.

Pfiffner, L., and H. Luka. 2003. Effects of low-imput farming systems on carabids and epigeal spiders - a paired farm approach. Basic Applied Ecology **4**:117-127.

Pinto, A. da Silviera., M. de Lana, C. Britto, B. Bastrenta, and M. Tibayrenc. 2000. Experimental *Trypanosoma cruzi* biclonal infection in *Triatoma infestans*: detection of distinct clonal genotypes using kinetoplast DNA probes. International Journal for Parasitology **30**:843-848.

Plague, G. R., and J. B. Wallace. 1998. Linkages between trophic variability and distribution of Pteronarcys spp. (Plecoptera: Pteronarcidae) along a stream continuum. American Midland Naturalist **139**:224-234.

Pollard, E. 1968. Hedges III. The effect of removal of the bottom flora of a hawthorn hedgerow on the Carabidae of the hedge bottom. Journal of Applied Ecology **5**:125-139.

Pollet, M., and K. Desender. 1986. Prey selection in carabid beetles (Col., Carabidae): Are diel activity patterns of predators and prey synchronized? Med. Fac. Landbouww. Rijksuniv. Gent **51**:957-971.

Pollet, M., and K. Desender. 1987. Feeding ecology of grassland-inhabiting carabid beetles (Carabidsae, Coleoptera) in relation to the availability of some prey groups. Acta Phytoph. Entom. Hung. **22**:223-246.

Schulz, K. L., and P. M. Yurista. 1995. Diet composition from allozyme analysis in the predatory cladoceran *Bythotrephes cederstroemi*. Limnology and Oceanography **40**:821-882.

Shelton, A. M., J.T. Andaloro and C.W. Hoy. 1983. Survey of ground-dwelling predaceous and parasitic arthropods in cabbage fields in upstate NY. Environmental Entomology **12**:1026-1030.

Sinitsyna, E. E., and S. Y. Chaika. 2003. Receptory organs of ground-beetle larvae. Entomologicheskae-Obozrenie **82**:276-288.

Solomon, M. G., J. D. Fitzgerald, and R. A. Murray. 1996. Electrophoretic approaches to predator-prey interactions. Pages 457-468 *in* W. O. C. Symondson and J. E. Liddell, editors. The Ecology of Agricultural Pests: Biochemical Approaches. Chapman and Hall, New York.

Suenaga, H., and T. Hamamura. 1998. Laboratory evaluation of carabid beetles (Coleoptera: Carabidae) as predators of diamondback moth (Lepidoptera: Plutellidae) larvae. Environmental Entomology **27**:767-772.

Suenaga, H., and T. Hamamura. 2001. Occurrence of carabid beetles (Coleoptera: Carabidae) in cabbage fields and their possible impact on lepidopteran pests. Applied Entomology and Zoology **36**:151-160.

Sunderland, K. D. 1987. A review of methods of quantifying invertebrate predation occurring in the field. Acta Phytopathologica et Entomologica Hungarica **22**:13-34.

Sunderland, K. D. 1988. Quantitative methods for detecting invertebrate predation occurring in the field. Annals of Applied Biology **112**:201-224.

Sunderland, K. D., N. E. Crook, D. L. Stacy, and B. J. Fuller. 1987. A study of feeding by polyphagous predators on cereal aphids using ELISA and gut dissection. Journal of Applied Ecology **24**:907-933.

Symondson, W., T. Gasull, and J. Liddell. 1999. Rapid identification of adult whiteflies in plant consignments using monoclonal antibodies. Annals of Applied Biology **134**:271-276.

Symondson, W. O. C. 2002. Molecular identification of prey in predator diets. Molecular Ecology **11**:627-641.

Symondson, W. O. C., D. M. Glen, M. L. Erickson, J. E. Liddell, and C. J. Langton. 2000. Do earthworms help to sustain the slug predator *Pterostichus melanarius* (Coleoptera: Carabidae) within crops? Investigations using clonal antibodies. Molecular Ecology **9**:1279-1292.

Symondson, W. O. C., and J. E. Liddell. 1993. A monoclonal antibody for the detection of arionid slug remains in carabid predators. Biological Control **3**:207-214.

Thiele, H. U. 1979. Relationships between annual and daily rhythms, climatic demands and habitat selection in carabid beetles. *in* T. L. Erwin, G. E. Ball, and D. R. Whitehead, editors. Carabid beetles: Their evolution, natural history, and classification. W. Junk, The Hague.

Tobolewski, J., M. J. Kaliszewski, R. K. Colwell, and J. James H. Oliver. 1992. Detection and identification of mammalian DNA from the gut of museum specimen ticks. Journal of Medical Entomology **29**:1049-1051.

Tooley, J. A., and R. J. Froud-Williams. 1999. Weed seed predation in arable field margins by carabid beetles (Carabidae:Coleoptera). Aspects of Applied Biology **54**:211-216.

Trougott, M. 1998. Larval and adult species composition, phenology and life cycles of carabid beetles (Coleoptera:Carabidae) in an organic potato field. European Journal of Soil Biology **34**:189-197.

Ulber, B., G. Stippich, and W. Wahmhoff. 1989. Possibilities, limitations and impact of supervised plant protection in arable farming III. Effects of different intensities of pesticide use on epigean arthropod predators in winter wheat, sugar beet and winter rape. Zeitschrift für Planzenkrankheiten und Planzenschutz **97**:263-283.

Wallin, H. P. A. C., B.S. Ekborn and A. Borg. 1992. Diet, fecundity and egg size in some polyphagous predatory carabid beetles. Entomologia Experimentalis et Applicata **65**:129-140.

Warner, D. J., l. J. Allen-Williams, A. W. Ferguson, and I. H. Willliams. 2000. Pest-predator spatial relationships in winter rape: implications for integrated crop management. Pest Management Science **56**:977-982.

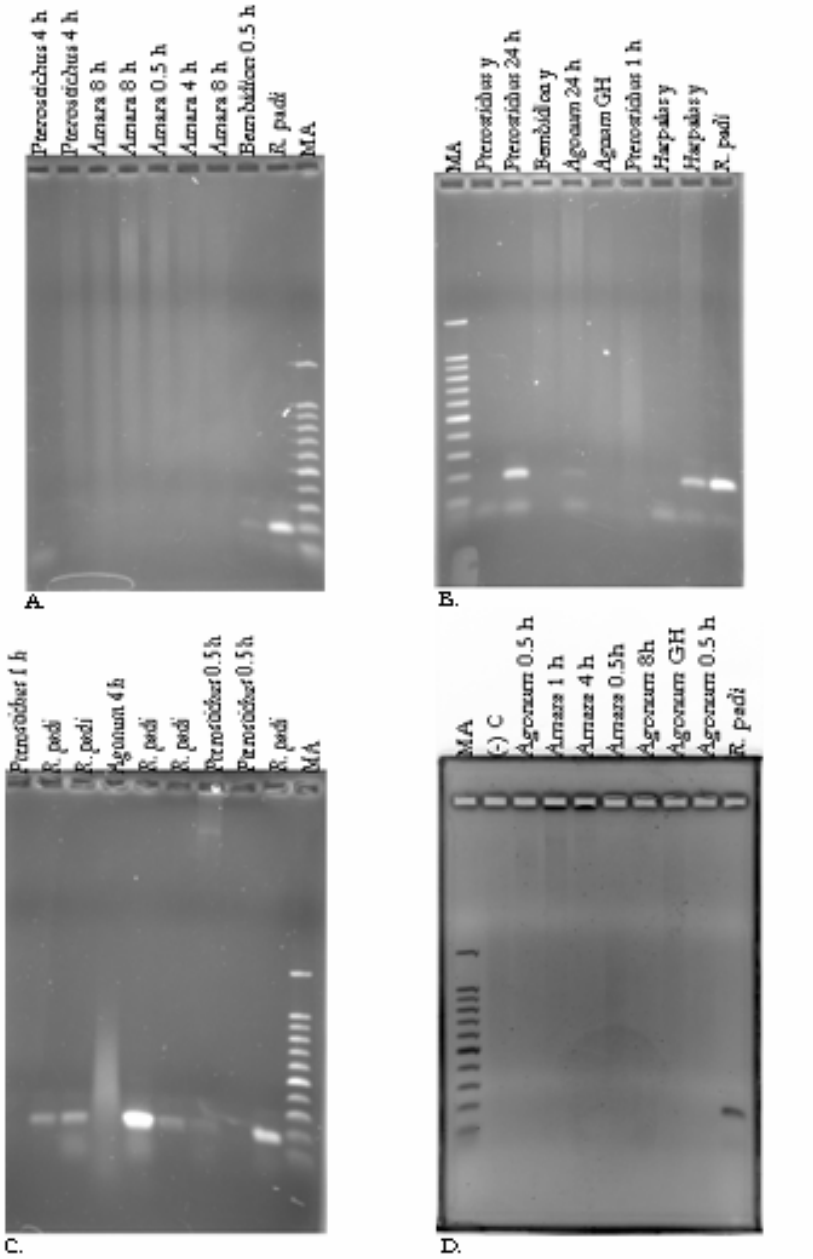
Winder, L. 1990. Predation of the cereal aphid *Sitobion avenae* by polyphagous predators on the ground. Ecological Entomology **15**:105-110.

Yi, S.-X., and T. S. Adams. 2001. Age and diapause-related acid and alkaline phosphate activities in the intestine and Malpighian tubules of the Colorado potato beetle, *Lepinotarsa decemlineata* (Say). Archives of Insect Biochemistry and Physiology **46**:152-163.

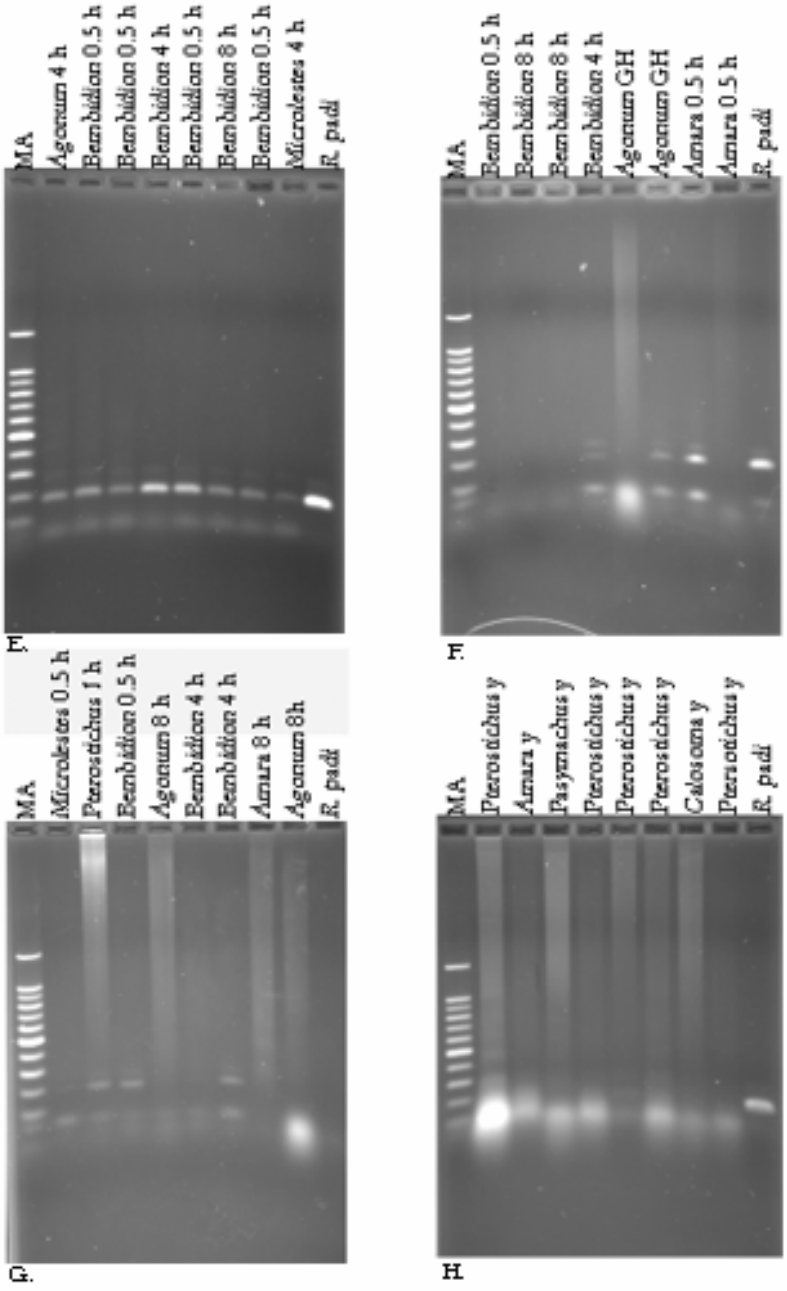
Zaidi, R. H., Z. Jaal, N. J. Hawkes, J. Hemingway, and W. O. C. Symondson. 1999. Can multiple-copy sequences of prey DNA be detected amongst the gut contents of invertebrate predators? Molecular Ecology **8**:2081-2087.

APPENDIX A

SAMPLE GELS



Figures A-D. 1.5% agarose gels showing gel electrophoresis seperation of 188-bp amplification products. MA is a 1500bp molecular marker. (-C) is water, h = time since ingestion is described in hours, GH = beetles tested from the greenhouse, y = beetles tested directly from the field. A laboratory fed bD. celtes test negative. B = field-collected *Harpalus* and laboratory-fed *Pterostichus* and *Amara* test positive. C. positive controls and a laboratory-fed *Pterostichus* test positive. D. Laboratory-fed *Agonum* test negative.



Figures E-H. 1.5% agarose gels showing gel electrophoresis seperation of 188-bp amplification products. MA is a 1500bp molecular marker. (-C) is water, h = time since ingestion is described in hours, GH = beetles tested from the greenhouse, y = beetles tested directly from the field. E. Smaller beetles test positive for 8h. F-G. GH-fed *Agonum* and laboratory fed beetles test positive. H. Low-molecular weight DNA streaks the lanes, but does not indicate a positive, compared to *R. padi* and molecular marker.