

Choosing natural enemies for conservation biological control: use of the prey detectability half-life to rank key predators of Colorado potato beetle

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Abstract

Determining relative strengths of trophic links is critical for ranking predators for conservation biological control. Molecular gut-content analysis enables ranking by incidence of prey remains in the gut, but differential digestive rates bias such rankings toward predators with slower rates. This bias can be reduced by indexing each predator's half-life to that of the middle-most half-life in a predator complex. We demonstrate this with data from key species in the predator complex of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), comprising adults and immatures of four taxonomically diverse species. These animals display order-of-magnitude variation in detectability half-life for the cytochrome oxidase I DNA sequence of a single CPB egg: from 7.0 h in larval *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae) to 84.4 h in nymphal *Perillus bioculatus* (Fabricius) (Hemiptera: Pentatomidae). The raw species-specific incidence of *L. decemlineata* DNA in the guts of 351 field-collected predators ranged from 11 to 95%, ranking them as follows: *C. maculata* adults < *Lebia grandis* Hentz (Coleoptera: Carabidae) adults < *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) adults < *P. maculiventris* nymphs < *P. bioculatus* adults < *P. bioculatus* nymphs. Half-life adjustment reorders the rankings: *C. maculata* adults < *P. bioculatus* adults < *P. bioculatus* nymphs < *P. maculiventris* nymphs < *L. grandis* adults < *P. maculiventris* adults. These changes in status demonstrate the value of half-life-adjusted molecular gut-content data for ranking predators. This is the first study to measure prey detectability half-lives for the key arthropod predators of a major insect pest, and to use them to evaluate the relative impact of all adults and immatures in this predator complex.

Introduction

Conservation biological control consists of 'premeditated actions for protecting and maintaining natural enemies' (Rabb et al., 1976). In practice, it comprises modifications

of crop cultural practices (Landis et al., 2000) and/or of pest management tactics (Gurr et al., 2000) to attract, arrest, and protect natural enemies. Most insect pests are attacked by a variety of natural enemies, not all of which are equally effective in pest suppression (Loreau et al., 2001; Straub & Snyder, 2006). This leads to the question of how one determines which specific natural enemies should be conserved, and, given several promising candidates, what is the relative return on investment for each species added (Cardinale et al., 2003). Agroecosystems can be surprisingly complex and speciose (Greenstone & Sunderland, 1999), making this a non-trivial question.

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Both the number and identity of species within a predator complex can determine the level of prey suppression (Root, 1973; Snyder et al., 2004; Ives et al., 2005). Predator feeding behavior can be complex (Sunderland, 1996, 1999; Juen & Traugott, 2005; Otto et al., 2008). For example, many predators are not strictly predaceous, acquiring nutrients from a variety of plant tissues, including pollen, seeds, and floral and extrafloral nectars, as well as honeydew (Lundgren, 2009). Further, the impact of plant resources on predator effectiveness may depend not only on their absolute availability, but also on their dispersion in the field and location on plants (Andow & Risch, 1985; Straub & Snyder, 2008). In mixed-cropping systems, the identity and arrangement of plants may also enhance or reduce the attractiveness of plant kairomones (Greenstone & Dickens, 2005). Finally, polyphagous predators tend to enter and remain in the system as long as alternate prey are available (Harwood et al., 2007; Birkhofer et al., 2008), but stenophages may fail to appear unless the pest is present in abundance (Chang & Kareiva, 1999; Symondson et al., 2002), making it difficult to attract what appear at face value to be excellent candidates.

Predation is the most difficult interspecific interaction to study in the field (Sunderland, 1988; Greenstone & Morgan, 1989). Of the available approaches (reviewed in Symondson, 2002), gut analysis is least disruptive to ecosystem processes: the field is visited intermittently, and collected predators are assayed in the laboratory. Many arthropod predators are fluid feeders, so a molecular approach is usually necessary to assess all interactions in a predator-prey system. Two technologies have dominated molecular gut-content analysis: serology, with monoclonal antibody-based assays predominating (Greenstone, 1996; Harwood et al., 2004), and polymerase chain reaction (PCR)-amplification of prey deoxyribonucleic acid (DNA) sequences (Symondson, 2002; Sheppard & Harwood, 2005; Garipey et al., 2007).

Because predators are trophically versatile, remains detected in the gut of a particular predator may not reflect predation by that predator on a live prey item, but rather scavenging on an already-dead animal, or it may reflect secondary predation on another predator that had killed and consumed that prey item (Harwood et al., 2001; Calder et al., 2005; Foltan et al., 2005; Juen & Traugott, 2005; Sheppard et al., 2005). Even when such 'food-chain errors' (Sunderland, 1996) are not an issue, so that an assay positive does represent predation, interpretation of assay data is not straightforward (Sopp & Sunderland, 1987). In the simple case where an assay positive represents the remains of a single prey item, the detectability of prey material within predators diminishes as it is digested (Hosseini et al., 2008, and references therein). Because the incidence

of positives in a field collection is an instantaneous snapshot of an hours-long feeding and digesting process, predator species with long detectability intervals for prey material will display a higher incidence of prey remains in the gut than those having shorter detectability intervals but identical feeding histories.

Exponential and binary regression models for decay of detectability with time since cessation of feeding on a standard meal provide good fits to molecular gut-content assay data (Greenstone & Hunt, 1993; Hagler & Naranjo, 1997; Chen et al., 2000; Ma et al., 2005; Greenstone et al., 2007; Fournier et al., 2008; Hosseini et al., 2008). Binary regression models are particularly appropriate for frequency data, such as those arising from gut-content analysis. Hence, the detectability half-life, defined as the time after which only half of the target meals can be detected in a cohort of predators as estimated by probit analysis, is an appropriate index of the detectability interval (Chen et al., 2000). Given a single-prey multiple-predator system, an index of each predator's half-life adjusted to that of the middle-most half-life in the predator complex can be used as a first-approximation adjustment to raw frequencies of prey remains in the guts of all predators. We illustrate this approach with a system in which a significant but manageable number of species and stages compose the complex of important predators of a single pest.

Several generalist and stenophagous arthropod predator species have been observed feeding on the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), in eastern North America (Ferro, 1994); of these, four were most abundant in our collections and are most prominent in the literature. The spotted pink lady beetle, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), is often the most abundant generalist predator on potato foliage (Benton & Crump, 1981; Hazzard et al., 1991; Hilbeck et al., 1997) and may kill more than half the *L. decemlineata* eggs in a generation (Grodén et al., 1990; Hazzard et al., 1991), as well as many small larvae. The spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), is a common generalist in potato (Hazzard et al., 1991; Heimpel & Hough-Goldstein, 1992; Ferro, 1994), consuming *L. decemlineata* eggs and larvae (Hough-Goldstein & McPherson, 1996; Tipping et al., 1999). The ground beetle *Lebia grandis* Hentz (Coleoptera: Carabidae) is an extreme specialist predator of eggs and larvae and an obligate parasitoid on prepupae of *Leptinotarsa* species (Chaboussou, 1939; Weber et al., 2006). The two-spotted stink bug, *Perillus bioculatus* (Fabricius) (Hemiptera: Pentatomidae), prefers *L. decemlineata* in the field (Knight, 1923), and is considered a specialist on exposed chrysomelid larvae (Heimpel, 1991). Like the two generalists, *L. grandis* and *P. bioculatus* are common in

potato fields (Hemenway & Whitcomb, 1967; Heimpel & Hough-Goldstein, 1992; Ferro, 1994).

The aims of this study were to determine the half-lives of DNA detectability for *L. decemlineata* in the guts of this suite of key predators of this pest, to demonstrate the use of these half-lives to weight the incidence of prey in the guts of field-collected predators, and to determine how this weighting affects the ranking of predators as biological control agents in a field situation.

Materials and methods

Field collections

Predators were collected from conventionally tilled potato fields in 2006 and 2007 as part of a larger study on the influence of habitat management on *L. decemlineata* biological control at the Beltsville Agricultural Research Center (BARC) in Beltsville, MD, USA (39°01'N, 76°55'W); complete details of potato management and experimental design are presented in Szendrei et al. (2010). Ten predator samples were taken between 26 May and 25 July in 2006, and eight between 4 June and 30 July in 2007, by hand-searching foliage. Predators were placed immediately in 75% EtOH at 4 °C in individual 5-ml glass vials that were stored until DNA extraction. We did not observe any regurgitation of gut contents into the EtOH.

Laboratory feeding studies

Prey insects. To minimize variability in half-life estimates, we used laboratory-reared animals. *Leptinotarsa decemlineata* originated from animals collected on potatoes at BARC in August 2007. The false potato beetle, *Leptinotarsa juncta* (Germar) (Coleoptera: Chrysomelidae), originated from animals collected on horsenettle (*Solanum carolinense* L., Solanaceae) in West Virginia pastures in 2003 and potato fields at BARC in 2004. Both colonies were maintained at the Invasive Insect Biocontrol and Behavior Laboratory at BARC, as described previously (Greenstone et al., 2007). *Leptinotarsa juncta* can co-occur in potato fields with *L. decemlineata*, either on potato or on *S. carolinense* on the margins of or within fields (Hemenway & Whitcomb, 1967). We used *L. juncta* and predators fed upon it as negative controls for all assays, and also to sustain normal predator metabolism during the feeding studies. Besides the two *Leptinotarsa* species and four predator species involved in the half-life experiments, we tested the *L. decemlineata* primers against 36 other potential alternate prey species collected from the study locality or known to occur in potato in the Eastern USA (Table 1).

To standardize the quantity of DNA consumed by each predator, we used a single egg of *L. decemlineata*, 24–48 h post-oviposition, as the prey item for half-life determina-

Table 1 Additional species tested for specificity against *Leptinotarsa decemlineata*-specific polymerase chain reaction primers **6**

Species	Collecting locality (all in USA)
ARANEAE	
Lycosidae	
<i>Pardosa milvina</i> (Hentz)	Beltsville, MD
<i>Rabidosa rabida</i> (Walckenaer)	Beltsville, MD
Theridiidae	
<i>Achaearanea tepidariorum</i> (CL Koch)	Ellicott City, MD
INSECTA	
Anthocoridae	
<i>Orius insidiosus</i> (Say)	Charleston, SC
Aphididae	
<i>Macrosiphum euphorbiae</i> (Thomas)	Beltsville, MD
Cerambycidae	
<i>Tetraopes tetrophthalmus</i> (Förster)	Beltsville, MD
Carabidae	
<i>Abacidus permundus</i> (Say)	Beltsville, MD
<i>Agonum striatopunctatum</i> Dejean	Beltsville, MD
<i>Agonum punctiforme</i> (Say)	Champaign, IL
<i>Amara aenea</i> (DeGeer)	Beltsville, MD
<i>Amara anthobia</i> A. Villa & G. B. Villa	Beltsville, MD
<i>Amara familiaris</i> (Duftschmid)	Beltsville, MD
<i>Amara cupreolata</i> Putzeys	Beltsville, MD
<i>Anisodactylus sanctaecrucis</i> (Fabricius)	Champaign, IL
<i>Bembidion affine</i> Say	Beltsville, MD
<i>Bembidion quadrimaculatum oppositum</i> Say	Beltsville, MD
<i>Bradycellus insulsus</i> (Casey)	Beltsville, MD
<i>Elaphropus anceps</i> (LeConte)	Beltsville, MD
<i>Elaphropus xanthopus</i> (Dejean)	Beltsville, MD
<i>Harpalus herbivagus</i> Say	Champaign, IL
<i>Harpalus fulgens</i> Csiki	Beltsville, MD
<i>Harpalus indigens</i> Casey	Champaign, IL
<i>Stenolophus dissimilis</i> Dejean	Beltsville, MD
<i>Stenolophus conjunctus</i> (Say)	Beltsville, MD
Coccinellidae	
<i>Coccinella septempunctata</i> L.	Beltsville, MD
<i>Epilachna varivestis</i> Mulsant	Beltsville, MD
<i>Harmonia axyridis</i> (Pallas)	Beltsville, MD
Lygaeidae	
<i>Geocorus punctipes</i> (Say)	Beltsville, MD
Pentatomidae	
<i>Euschistus servus</i> (Vollenhoven)	Beltsville, MD
<i>Oebalus pugnax</i> (Fabricius)	Beltsville, MD
Thripidae	
<i>Franklinella occidentalis</i> (Pergande)	Charleston, SC

tion. Eggs were separated carefully from masses oviposited on potato foliage.

Predatory insects. With the exception of *L. grandis*, all predators used in this research were derived from laboratory colonies, to provide experimental cohorts of known

age, environmental conditions, and dietary history. Establishment and maintenance of the *C. maculata* colony was described previously (Greenstone et al., 2007). The *P. maculiventris* colony originated with adults collected from alfalfa in Boone County, MO, USA, in the summer of 2000, and had been maintained continuously for ca. 120 generations by the protocol of Coudron et al. (2002) at the time of the experiment. The *P. bioculatus* colony originated from adults collected from potato fields at BARC in the summer of 2007, and had been maintained continuously for ca. 15 generations by the protocol of Coudron & Kim (2004); both pentatomids were reared at the Biological Control of Insects Research Laboratory in Columbia, MO, USA, on excess quantities of coddled fourth instars of the cabbage looper, *Trichoplusia ni* (Hübner) (Wittmeyer et al., 2001; Coudron et al., 2002). Early third instars collected within 8 h of molting, or unmatated adults collected 5 days after eclosion, were shipped at 6 ± 1 °C (Coudron et al., 2007) from Missouri to Maryland, where all feeding studies were performed. The animals were provided with water but deprived of food during the 48-h shipment; feeding studies were started upon arrival in Beltsville.

Adult *L. grandis* were collected by hand on 8 August 2007 from potato fields at BARC and placed in group cages with water wicks and maintained on *L. juncta* larvae for 3 weeks prior to the feeding experiments.

Feeding protocol. Details of the feeding protocol to determine detectability half-lives for *L. decemlineata* DNA were given previously (Greenstone et al., 2007). Briefly, to ensure that the animals were sufficiently hungry to feed, they were starved before the experiment for 24 h (48 h for *P. maculiventris* and 120 h for *L. grandis*, because they would not feed readily at 24 h), transferred to individual Petri dishes with water wicks, and placed overnight in an incubator programmed to simulate mean hourly temperatures 15 cm above the soil surface and appropriate photoperiod for late May (BARC, 2003; feeding under field conditions is imperative because half-lives are temperature-sensitive (Hagler & Naranjo, 1997; Von Berg et al., 2008). Feeding trials were begun the following day at 10:00 hours. Except for *L. grandis*, which lacks free-living larvae, separate trials were performed for adults and third instars.

Each predator was fed a single *L. decemlineata* egg and observed until it had consumed it; those that had not consumed the egg within 2 h were dropped from the experiment. With the exception of adult *L. grandis*, which had to be hand-collected, and nymphal *P. maculiventris*, because the *Podisus* colony was lost in a laboratory accident before sufficient animals were obtained, 20 individuals of each species were harvested at 4-h intervals beginning with ces-

sation of feeding as time $t = 0$ and ending at $t = 20$ h. Preliminary analysis showed that this was insufficient for the two pentatomid species, so additional groups of 20 animals were run at 24-h intervals out to 96 or 120 h. The numbers of *L. grandis* adults and *P. maculiventris* nymphs run at each interval are given in Table 2.

Animals in the $t = 0$ h groups were frozen at -20 °C immediately after consuming the *L. decemlineata* egg. For those designated for later time points, any remaining *L. decemlineata* egg chorion was removed, 3–5 *L. juncta* eggs were provided, and the animal was returned to the incubator. At its designated time since feeding, each remaining predator was removed from the incubator and frozen at -20 °C until molecular assay.

DNA procedures. DNA was extracted and purified per Greenstone et al. (2005); field-collected animals were removed from the EtOH into which they had been collected, blotted on tissues, and air-dried before extraction. Protocols for preliminary and species-specific PCRs, species-specific PCR primer design, and agarose gel electrophoresis were provided in Greenstone et al. (2007); primer sequences, annealing temperatures, and amplicon sizes are given in Table 3. Each PCR reaction included two positive (single *L. decemlineata* egg) controls, two positive fed-predator ($t = 0$) controls, two each of three kinds of negative controls – *L. juncta*, *L. juncta*-fed predator, and starved predator – and one no-DNA control. Additionally, control reactions utilizing the same PCR cocktail and plates were run simultaneously with all samples to verify that the DNA in the samples was amplifiable: for the half-life experiments these were the predator-specific reactions; control reactions for the field samples employed generic cytochrome oxidase I primers (Simon et al., 1994).

Statistical analysis. For each species and stage combination, the half-life for DNA detectability and its 95% fiducial limits were determined with the two-parameter probit

Table 2 Numbers of *Podisus maculiventris* nymphs and *Lebia grandis* adults run at each time interval

Interval (h)	No. <i>P. maculiventris</i>	No. <i>L. grandis</i>
0	20	12
4	20	15
8	17	11
12	20	12
16	13	9
20	20	
48	20	
72	20	

Table 3 Primer sequences

Species	T _a	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Size
Prey				
<i>Leptinotarsa decemlineata</i>	54	Cpb5S – CCTTTTCTCTTGGGCAGTTAT	Cpb6A – TTATCCCAAAATCCAGGTAGAAT	214
<i>Leptinotarsa juncta</i>	53	FpbWV51 – CACTGTCAATCAAAATATTGCCCAT	FpbWVA1 – TGCTAAACGGGGAGGGGA	219
Predators				
<i>Coleomegilla maculata</i>	53	Cmac1S – ACAGTTTATCTCCCTTATCCTCTA	Cmac2B – TTTTTCCTGCTTCTTGTAGTGAAT	414
<i>Lebia grandis</i>	59	Lg3S – AAGACTAGTTGAAAGAGGAGCAGGTA	Lg3A – TCCAAACAGATCAAAACAAATAAAGGTA	223
<i>Perillus bioculatus</i>	60	Per1S – ATG CGC CCA ATA GGA AT	Per1A – TTG ATA ATA CAT AGT GGA AGT GG	633
<i>Podisus maculiventris</i>	55	Pod2S – GATCAGTTGGAATTACCGCTTTAT	Pod2A – TTGGATTTTATGTCACCTTAGGGTTTA	683

T_a, annealing temperature (°C); size, size of amplicon in bp.

model (Proc PROBIT; SAS Institute, 1999). Species-stage combinations whose 84% fiducial limits do not overlap are statistically different at P<0.05 (Payton et al., 2003).

Half-life adjustment of Leptinotarsa decemlineata incidence in the gut. For each species-stage combination, the proportion positive for *L. decemlineata* DNA of field-collected animals was substituted into the probit regression equation, derived from the laboratory feeding study for that species and stage. The regression was then solved to obtain the time since feeding, i.e., the value of the explanatory variable required to obtain that observed percentage. This quantity was then substituted into the probit regression equation as the explanatory variable for that species-stage combination displaying the middle-most half-life among all of the species-stage combinations to arrive at an adjusted observed proportion. In effect, we ask what proportion positive each species-stage would exhibit if their half-lives all equaled that of the middle-most species-stage combination. Choice of the middle-most half-life was arbitrary: any species-stage combination could be used as the group to which adjustments were made and produce the same rankings.

Results

The *L. decemlineata* primers exhibited perfect specificity, amplifying DNA of the target species but none of other species in the experiment or potentially present in the field. *Leptinotarsa decemlineata* eggs and/or larvae were present in the field on all sampling dates (Szendrei et al., 2010). Of 351 predators collected from the field, 46% had detectable *L. decemlineata* DNA in the gut, with the incidence ranging from 11% in *C. maculata* adults to 95% in *P. bioculatus* nymphs (Table 4). DNA digestive rates varied greatly, with

Table 4 Molecular gut-content analysis of field collected predators, with 2006 and 2007 samples combined. Numbers are individual predators hand-collected from conventionally tilled potato plots at the Beltsville Agricultural Research Center. Positives are individuals positive by polymerase chain reaction for *Leptinotarsa decemlineata* cytochrome oxidase I DNA

Species	Life stage	Total	Positives	Proportion
<i>Coleomegilla maculata</i>	Adult	141	15	0.11
	Larva	2	0	0
<i>Lebia grandis</i>	Adult	56	18	0.32
<i>Perillus bioculatus</i>	Adult	58	49	0.85
	Nymph	41	39	0.95
<i>Podisus maculiventris</i>	Adult	44	33	0.75
	Nymph	9	7	0.78
Total		351	161	0.46

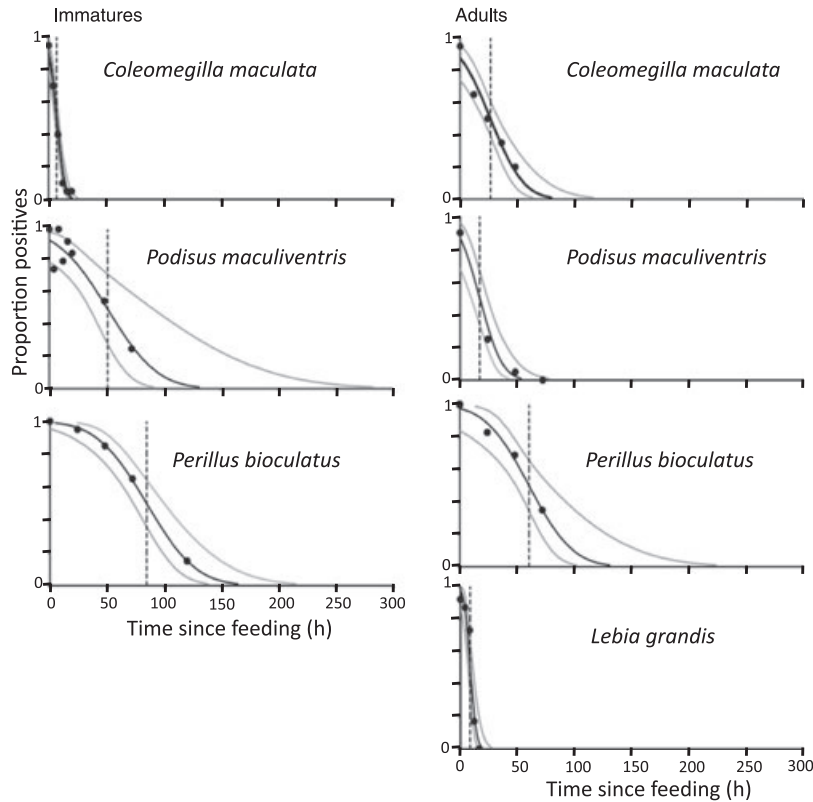


Figure 1 Results of *Leptinotarsa decemlineata* DNA half-life studies for immature and adult predators. Regressions and 95% fiducial limits were fitted by a two-parameter probit model (Proc PROBIT; SAS Institute, 1999). The dotted vertical line indicates the half-life.

Table 5 Results of half-life analysis for detectability by polymerase chain reaction of cytochrome oxidase I DNA consumed from a single egg of *Leptinotarsa decemlineata* in predators held under simulated field conditions

Species/stage	Half-life (h)	95% fiducial limits		Intercept	Slope
Immature					
<i>Coleomegilla maculata</i>	7.0e	5.2–8.6	1.2776	–0.1820	
<i>Lebia grandis</i>	—				
<i>Perillus bioculatus</i>	84.4a	72.8–99.7	2.4583	–0.0291	
<i>Podisus maculiventris</i>	50.9b	35.5–90.8	1.4824	–0.0291	
Adult					
<i>Coleomegilla maculata</i>	26.4c	19.9–33.3	1.1618	–0.0440	
<i>Lebia grandis</i>	8.6e	6.7–10.7	1.9453	–0.2274	
<i>Perillus bioculatus</i>	60.5b	49.1–78.6	2.0693	–0.0332	
<i>Podisus maculiventris</i>	17.1d	9.7–23.8	1.0276	–0.0581	

Half-lives were determined with a two-parameter probit model (Proc PROBIT; SAS Institute, 1999). Half-lives followed by different letters are statistically different (Payton et al., 2003).

Immature *Coleomegilla maculata* and *Podisus maculiventris* half-lives from Greenstone et al. (2007), by permission.

the pentatomids tending to be slower (Figure 1). The half-lives of the predators and their stages ranged from 7.0 h in larval *C. maculata* to 84.4 h in nymphal *P. bioculatus* (Table 5), indicating that the probability of collecting a *P. bioculatus* nymph containing detectable *L. decemlineata* cytochrome oxidase I DNA is greater than that for a *C. maculata* larva with identical feeding history. The half-lives of adults and immatures within each species were statistically different; half-lives of all adults were also statistically different from one another, as were those of all immatures (Table 5). The data fit the probit model well, with $P < 0.001$ in all cases. The raw incidences were adjusted as described above by indexing them to that of *P. maculiventris* nymphs, which was the species-stage combination whose detectability half-life was nearest the mean half-life of all species and stages studied (Table 6).

When the raw incidence values are used, the members of the predator complex rank thus: *C. maculata* adults < *L. grandis* adults < *P. maculiventris* adults < *P. maculiventris* nymphs < *P. bioculatus* adults < *P. bioculatus* nymphs (no target DNA was detected in *C. maculata* larvae). Adjusted incidences change the rankings for the members of the same complex as follows: *C. maculata*

Table 6 Incidence (%) of cytochrome oxidase I DNA detected by polymerase chain reaction assay in predators collected from conventionally tilled potato plots at the Beltsville Agricultural Research Center in 2006 and 2007 (combined). For each species, the raw incidence was used to calculate the corresponding time since feeding from its probit model, which was then entered into the probit model for *Podisus maculiventris* nymphs to calculate the adjusted incidence

Predator	Incidence (%)		
	Life stage	Raw	Adjusted
<i>Coleomegilla maculata</i>	Adult	10.6	39.4
	Larva	0	0
<i>Lebia grandis</i>	Adult	32.1	88.0
<i>Perillus bioculatus</i>	Adult	84.5	73.0
	Nymph	95.1	75.2
<i>Podisus maculiventris</i>	Adults	75.0	90.4
	Nymph	77.8	77.8

adults < *P. bioculatus* adults < *P. bioculatus* nymphs < *P. maculiventris* nymphs < *L. grandis* adults < *P. maculiventris* adults. The adjusted rankings place adults of the polyphagous pentatomid *P. maculiventris* first, ahead of the stenophagous carabid *L. grandis*. This is a dramatic change from the original rankings based on raw incidence (Table 4), which placed all stages of the two pentatomids first, with the stenophagous *P. bioculatus* before the polyphagous *P. maculiventris*.

Discussion

Molecular gut-content assay has revolutionized our ability to determine the role of predators in suppressing insect pest populations. Arranging the members of a predator complex by incidence of a pest in the gut is a reasonable first step in ranking them for conservation biological control (Birkhofer et al., 2008; Fournier et al., 2008). For field-collected predators of *L. decemlineata*, ranking by raw *L. decemlineata* DNA incidence places both stages of *P. bioculatus* first, followed by both stages of *P. maculiventris*, adults of *L. grandis*, and adults of *C. maculata*. However, when the incidence is adjusted for predator-specific differences in prey detectability half life, *P. maculiventris* adults move into first place, followed by *L. grandis* adults, *P. maculiventris* nymphs, both stages of *P. bioculatus*, and adults of *C. maculata*. The most striking change is the elevation of *L. grandis* from next-to-worst to next-to-best candidate. It is also notable that the adjusted values rank as the two best candidates a polyphagous hemipteran and a stenophagous coleopteran, suggesting that degree of pest species-specificity may not be the best predictor for the effectiveness of a natural enemy.

Our half-life adjustment does not account for differences in feeding pattern amongst predator species over time of day, nor for repeat feeding on the same prey before a prior prey item becomes essentially undetectable. If, for example, *P. maculiventris* nymphs are apt to eat another egg before the first becomes undetectable, its detectability half-life will appear lengthened (Naranjo & Hagler, 1998). The impact of feeding interval on the adjusted incidence cannot be determined without knowing the feeding frequency of all predators in the complex. The attack rate of adult female *P. maculiventris* on *L. decemlineata* larvae but not eggs has been measured in the field, giving a feeding interval of roughly 44 h (after O'Neil, 1997). However, attack rates have not been measured in the field for the other members of this predator complex. Finally, consumption of alternate prey species would increase feeding intervals for *L. decemlineata* meals, an effect that would be smaller for the more stenophagous species, *L. grandis* and *P. bioculatus*, than for those with broader diets.

There are several outstanding technical issues in the application of molecular gut-content assay data to decision-making in biological control. One is determining whether prey remains entered the gut through predation or via one or more alternative food-chain pathways. Molecular gut content assays may detect scavenging or secondary predation, making it necessary to estimate the likelihood of such events for each system under study. The probability of scavenging will depend upon each predator's innate preference for live vs. dead prey, availability of cadavers, and their state of decay upon encounter by the predator (Foltan et al., 2005). It might be possible to detect the presence or absence of enzymes found in living animals that are rapidly degraded in cadavers, but this will require fundamental research to discover which enzymes behave in this fashion (Juen & Traugott, 2005). Whether or not secondary predation is likely to be an issue depends on the system under study and the sensitivity of the assay (Harwood et al., 2001; Sheppard et al., 2005).

The quantity of prey protein or DNA in the gut can be estimated by ELISA or quantitative PCR. This quantity may decay with time (Fichter & Stephen, 1981; Sopp & Sunderland, 1987; Harwood et al., 2001; Fournier et al., 2006; Weber & Lundgren, 2009), enabling estimation of a half-life for prey remains (Symondson & Liddell, 1995). This is not, however, the same as the detectability half-life: the latter's application to gut-content data analysis provides a predator-species-specific standard of comparison for the value of a positive by removing the bias that would otherwise overvalue the role of species with longer detectability periods (Sopp et al., 1992; Chen et al., 2000; Fournier et al., 2006; Hosseini et al., 2008).

The data from standard molecular gut-content assays, whether serological or DNA-based, are positives and negatives. The positives, however, subsume unknown variables that affect the detectability of the target molecule. These include the number and size of prey eaten, the stage of prey eaten, the time since consuming a prey item, the temperature regime from the time of consumption until the animal was collected, and whether alternate prey were consumed during the feeding interval (Sopp & Wratten, 1986; Sopp et al., 1992; Hagler & Naranjo, 1997; Weber & Lundgren, 2009). The largest component of prey size is prey stage, which may be marked by stage-specific proteins (Goodman et al., 1997; Fournier et al., 2006); this has made it possible to identify prey stage-specific predation in the field by immunoassay (Sigsgaard et al., 2002; Fournier et al., 2008). DNA-based assays cannot distinguish stages. Reverse-transcriptase (RT-) PCR could do so, but this would require a major research effort to identify stage-specific proteins and the RNA sequences encoding them. Provided the stage consumed can be determined, the number of individuals consumed might be estimated by RT-PCR (Nejstgaard et al., 2007; Zhang et al., 2007; Weber & Lundgren, 2009). Alternatively, the mean number of prey items in the gut represented by a sample of gut-content assay positives could be estimated by applying the Poisson distribution (Nakamura & Nakamura, 1977; Greenstone, 1979; Lister et al., 1987), but this alone would not translate to a rate of feeding without knowledge of the feeding interval and size of the prey item.

Finally, by making use of the observation that shorter DNA fragments tend to become undetectable more slowly than longer ones (Agustí et al., 1999; Zaidi et al., 1999; Chen et al., 2000). Hoogendoorn & Heimpel (2001) showed that one may determine the time since feeding by using several sets of PCR primer pairs that target fragments of different lengths. In at least one case, however, this relationship between amplicon size and digestive rate did not obtain (Juen & Traugott, 2005), suggesting that this approach may be taxon- or temperature-dependent. Combining all of these factors into a true quantitative, i.e., per capita, estimate of predator impact on a pest will require a significant modeling effort; the success of previous attempts to model various aspects of predation from gut-content data provides grounds for optimism (Sopp et al., 1992; Naranjo & Hagler, 2001).

To study multiple predator effects empirically, one should not disturb the real-life interactions that characterize a diverse species community. Molecular gut-content analysis enables assessment of feeding by an entire predator complex in its unmanipulated environment. Given a sufficiently robust model for deriving quantitative estimates of feeding rate, gut content analysis

can support estimates of the contribution of each predator in a complex to the suppression of the pest, and thereby identify those species and stages most worthy of conservation. For example, the 'sampling effect' (Tilman, 1999; Loreau et al., 2001), where a dominant predator affects prey abundance, may be revealed by molecular gut content analysis. In our example, half-life adjustment of the raw incidence of *L. decemlineata* DNA in the gut revealed *P. maculiventris* and *L. grandis* to be more effective biological control agents of *L. decemlineata*, on a per capita basis, than the other species in the complex, suggesting that conservation management practices should target them.

We used a realistic but simple predator-prey system to develop these ideas. By using multiple DNA primers for predators and prey present in more complex systems, entire networks of intraguild predators and alternate prey can be mapped (Agustí et al., 2003; Harwood et al., 2007; Saccaggi et al., 2008). The results would provide additional insight into the mechanisms of biological control, and aid managers tasked with modifying cultural methods to enhance biocontrol of pests. The cost-effectiveness and relative ubiquity of molecular laboratories allows for the quick development of PCR primers for prey and predators; primers for cosmopolitan predator-prey systems can also be shared among laboratories, further facilitating the use of these methods (Symondson, 2002; Harwood & Greenstone, 2008).

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