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Changes in digestive rate of a predatory beetle over its larval stage: Implications for dietary breadth

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ABSTRACT

Prey and non-prey foods differ substantially in their suitability for zoophytophagous omnivores, but the relative quality of these foods depends on the stage-specific digestive capabilities of the organism in question. Quantitative (or real-time) PCR was used to amplify food-specific DNA and measure consumption rates and digestion efficiencies of four foods – two prey (*Aphis glycines* and *Leptinotarsa decemlineata* eggs) and two non-prey (*Zea mays* pollen and the yeast *Saccharomyces cerevisiae*) species – over different larval stages of *Coleomegilla maculata*. The amount of *Z. mays* pollen consumed increased as larvae aged, but not proportionately with larval size, such that consumption rates decreased uniformly with insect age. While aging larvae fed *A. glycines* had a similar pattern in their diminishing consumption rates, they consumed similar amounts of *A. glycines* regardless of age, suggesting a negative feedback mechanism for consumption of this species of aphids. Older larvae digested three of the four foods significantly more efficiently than younger larvae, the exception being larvae fed *A. glycines* which was digested at a similar rate throughout the larval stage. There was a significant effect of time on food quantity detected for all four species of food. We conclude that *C. maculata* expands its physiological capacity for digesting prey and non-prey foods as they age in order to better accommodate the increased nutritional needs of the older larvae. This strategy has important implications for the life history strategies of zoophytophagous insects and how they function within foods webs.

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1. Introduction

Foods differ in their suitability for an organism, and this simple fact has important implications that ultimately mediates food web interactions, community structure, and ecosystem processes. The relative quality of different foods for select life stages of an animal has been determined for a wide range of trophic guilds represented by diverse animal species, including insects. But the nutritional requirements of an organism change (often dramatically) over its life (Crailsheim et al., 1992; Wikelski et al., 1993; Barton Browne, 1995), and this added complexity needs to be accounted for as a conceptual framework for nutritional ecology is developed (Raubenheimer et al., 2009). Among insects, differences in feeding behavior between life stages are well documented (e.g., Michaud, 2005), but how food suitability and dietary breadth changes within a life stage of an insect remains poorly understood (Scriber and Slansky, 1981; Barton Browne, 1995). Changes in dietary breadth within a life stage become particularly important for zoophytophagous insects, whose presumed ecological role within a food

web is that of predator, but who in reality consume numerous non-prey resources that affect the strength of their interactions with prey (Hagen, 1986; Coll and Guershon, 2002; Wäckers, 2005; Hunter, 2009; Lundgren, 2009a).

There are two main approaches for studying food consumption, utilization, and the relative suitabilities of different foods for animals (Waldbauer, 1968). First, direct or gravitational methods that weigh the amount of food ingested, weight gained by the organism, and amount of feces produced are useful for estimating consumption and the efficiency at which a species converts ingested material into biomass (Waldbauer, 1968). Another less labor-intensive approach for assessing a food's suitability is the use of digestive rates of markers (such as chromic oxide or radioisotopes) fed alongside a meal (McGinnis and Kasting, 1964; Warner, 1981; Wikelski et al., 1993; Afik and Karasov, 1995). Retention times of a marker are often well correlated with enzymatic processing of a food item in the gut and thus retention times represent the relative digestibility of different foods (Warner, 1981; Afik and Karasov, 1995; Chown and Nicolson, 2004). But these markers are seldom truly integrated throughout a meal (i.e., they only coat the surface of a meal and thus are consumed at differential rates as a meal is ingested), especially in natural foods like prey. Waldbauer (1968) contended that the use

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of a “naturally occurring constituent of the food would be an ideal marker”, but there has been little use of this type of marker system within the eco-physiology literature. The relative suitability of a food item is often reflected in an insect's behavioral and physiological responses to it. When faced with food of poor nutritional content, an insect can (1) consume more of it to obtain more of the limiting nutrients (compensatory consumption), (2) consume other foods with different nutrition (altered dietary breadth), and (3) change physiologically to digest the poor quality food more efficiently (digestive compensation) (Chown and Nicolson, 2004).

A tool that is routinely used to investigate predator–prey relationships is genetic gut content analysis (GCA) of the predator using polymerase chain reaction (PCR) primers that amplify unique regions of the prey's genome (Symondson, 2002; Greenstone et al., 2007; Harwood et al., 2007; Harwood et al., 2009; Lundgren et al., 2009a; Weber and Lundgren, 2009a,b), but this technique has not heretofore been used to estimate nutritional suitability of foods. Predators digest these DNA markers over time, and the rate at which this occurs is dependent on a range of environmental factors and inherent qualities of the food itself (Zhang et al., 2007; Durbin et al., 2008; Weber and Lundgren, 2009b). The complexities of this digestion process hamper interspecies comparisons of predator efficacy and predation rates under field conditions. However, GCA of a “naturally occurring constituent of the food” (*sensu* Waldbauer; i.e., the food's DNA) is a potentially useful technique for understanding how digestive capabilities change ontogenetically within a single predator species. By measuring the quantity of food DNA consumed per unit of time, quantitative PCR (qPCR) can measure whether an insect changes its consumption rate of a given food over its life (e.g., compensatory consumption, possibly due to low nutrient foods). Also, qPCR can be used to measure the relative digestion rates of a given food's DNA over an insect's larval life, as an indication of how the digestive capabilities of that insect change.

The objective of this study was to determine how a zoophytophagous insect's capability for digesting prey and non-prey foods changes over the course of its larval life. *Coleomegilla maculata* (Coleoptera: Coccinellidae) is a widespread and abundant lady beetle of economic importance as a predator of a variety of agricultural pests (Evans, 2009; Obyrcki et al., 2009). Many coccinellids rely on non-prey foods in addition to prey under field conditions (Lundgren, 2009b), and *C. maculata* frequently consumes pollen and fungal spores as part of its natural diet (Lundgren et al., 2004; Lundgren et al., 2005). In fact, it can complete its development and reproduce on a diet consisting solely of pollen, although this food is inferior in quality to preferred prey (Smith, 1960; Hodek et al., 1978; Lundgren and Wiedenmann, 2004; Michaud and Grant, 2005), due in part to sterol limitation of the pollen (Pilorget et al., 2010). Previous research has shown that the ability of *C. maculata* larvae to convert pollen into biomass improves as they age (Lundgren and Wiedenmann, 2004). But it is unknown whether this phenomenon is pollen-specific, or if it applies to their ability to digest other foods and thus represents a potential physiological or behavioral expansion of dietary breadth with larval age.

Using qPCR-based GCA and laboratory feeding assays, the consumption and digestion rates of two prey species (*Aphis glycines* and *Leptinotarsa decemlineata* eggs) and two non-prey foods (*Zea mays* pollen and the yeast *Saccharomyces cerevisiae*) by four successive instars of *C. maculata* were measured. With this information, hypotheses were tested that (1) *C. maculata* of varying ages consume similar quantities of a given food per unit of body mass during a set amount of time (i.e., does not display compensatory consumption), and (2) *C. maculata* larvae digest each food item at a similar rate throughout larval development

(i.e., there is not a change in their digestive capability with age). The results are discussed in the context of the nutritional ecology and dietary breadth of *C. maculata*.

2. Methods

2.1. Study organisms

C. maculata was collected from cropland in Beltsville, MD, and was kept in culture for 4 mo before use in experiments. Larvae were reared to the desired stadium on a 50:50 mixture (by weight) of Bee Pro pollen substitute (Mann Lake Ltd., Hackensack, MN) and dried freshwater amphipods (*Gammarus lacustris*, Tetra Holding, Inc., Blacksburg, VA). This diet has proven excellent for rearing *C. maculata* through several generations in the laboratory. *A. glycines* Matsumura (the soybean aphid) was collected from infested soybean fields in Brookings, SD, and was reared in a growth chamber on *Glycines max* L. var. Surge SD(M)92-1233 (South Dakota State University, Brookings, SD) for at least 4 mo prior to the assays. Aphids were removed from soybean foliage using a fine paintbrush and placed directly into the feeding arenas described below. *L. decemlineata* Say (Colorado potato beetle) were collected from potato fields in Beltsville, MD, and maintained in culture on potato plants for at least 3 mo (3–5 generations) before the assays. Three-day-old egg clutches were separated from the potato foliage using a fine paint brush, and placed individually into randomly selected feeding arenas. Corn (*Z. mays* L.; NK4242, Northrup King Company, Golden Valley, MN) plants were grown in 7.6-l pots (two plants per pot) in the greenhouse until anthesis. Climatic conditions were 27 °C, approximately 40% relative humidity, and a photoperiod of 14 hr daylight. Plants were watered twice daily, and received fertilizer (20–20–20, N–P–K) weekly. When the tassels were evident, pollen was collected, sieved (55.6 openings/cm), and stored according to protocols outlined by Pilorget et al. (2010). *S. cerevisiae* Meyen ex. E. C. Hansen was obtained commercially (Nottingham Brewing Yeast, Danstar Ferment AG, Zug, Switzerland). This food was selected to represent fungal food, which microscopic gut content analyses have shown is a frequent food source for *C. maculata* under field conditions (Forbes, 1883, Putman, 1964). The four food types were selected based on the known dietary records of *C. maculata* under field conditions, including direct feeding observations and gut content analyses of field collected specimens.

2.2. Feeding assays

Independent assays were conducted for each food. *C. maculata* larvae were held individually in 4 cm diam. plastic Petri dishes, and were provided only water (as a saturated cotton wick) for 24 h prior to the assay. At 07:30 on the days of the assays, excess food of each type was placed into clean 4 cm diam. dishes ($n > 70$ each for each instar). An individual larva was placed into each dish and then observed (microscopically in the case of pollen and yeast) until it began to feed. For treatments fed *A. glycines* and *Z. mays* pollen, each individual was allowed to feed for 5 min without interruption. In the case of *A. glycines*, additional prey items were hand-fed to larvae using a fine paintbrush if they completed their initial meal within 5 min. Larvae were allowed to consume a single *L. decemlineata* egg, except for fourth instars, which each received three *L. decemlineata* eggs. Instars were allowed to consume *S. cerevisiae* for varying amounts of time, ranging from 10–20 min. After feeding, larvae were transferred to a clean Petri dish, and were randomly assigned an execution time. Between 10–12 individuals were killed at 0, 0.5, 1, 2, 3, 4, and 6 h post-feeding by placing them into pre-chilled 70% EtOH, which was then frozen at –20 °C until gut contents could be analyzed.

2.3. Gut content analysis

DNA was extracted from individual *C. maculata* larvae using DNeasy Blood & Tissue extraction kits (#69506, Qiagen, Valencia, CA, USA). Samples (i.e. macerated whole, individual larvae) were incubated in ATL buffer with Proteinase K for 3 h. Food-specific primer sets were either obtained from published literature, or were designed new using the PrimerSelect feature of Lasergene 7.0.0 software (DNASTAR Inc, Madison, WI, USA) and sequences present in Genbank. The amount of food DNA present in each *C. maculata* larva was quantified using qPCR on a Stratagene MX3000P thermocycler (Stratagene, La Jolla, CA, USA) in 25 µl reactions with the following ingredients: 8 µl PCR-grade water, 12.5 µl Quantitect SYBR Green PCR Master Mix (#204143, Qiagen), 1.5 mM BSA solution (Roche Diagnostics GmbH, Mannheim, Germany), 2 µl template DNA (approximately 1–88 µg; assessed using absorbance ratio produced at 260/280 nm), and food-specific forward and reverse primer sets. Amplification conditions were a single cycle of 95 °C, followed by 50–75 cycles (number of cycles applied to each food are listed in Table 1) of 94 °C for 15 s, 52–56 °C (see Table 1) for 30 s, and 74 °C for 30 s. To ensure that our PCR was amplifying the correct food's DNA, a food-specific dissociation (melting) temperature was determined for each PCR product by incubation at 95 °C for 60 s, and then recording the fluorescence every 0.5 °C from 55 to 94 °C.

The result of qPCR is a threshold cycle (Ct) of the PCR in which the fluorescence of the product can be detected above background fluorescence. The amount of food's DNA in each sample is thus related to the number of PCR cycles that occur before threshold fluorescence is reached. Each food category was analyzed in separate PCR series, but larvae of all stadia were randomly assigned to plates within a food category. Five wells of a single positive control sample were run on each 96-well plate, and the absolute deviation of this series from the inter-plate mean Ct was subtracted from each well's value to standardize plate-to-plate variability in the PCRs. These controls were extractions of the following material: *L. decemlineata*, three 3-d-old eggs, 0.022 g *Z. mays* pollen, 0.021 g *S. cerevisiae*, and 0.003 g of *A. glycines*. Additionally, at least three wells on each plate were devoted to unfed *C. maculata* DNA, and three wells to no-template-controls.

2.4. Data analysis

The Ct values from each plate were standardized according to the mean deviation of the positive control series on that plate from the overall (inter-plate) mean of the positive control samples. For each value in which the Ct of a sample exceeded the number of PCR cycles in our assay, a randomly selected value between the highest Ct recorded for an instar/treatment combination and the maximum number of cycles run was generated. For each individual fed *A. glycines* or *Z. mays* pollen, a consumption index was calculated as the initial meal size (Ct⁻¹ at t = 0), divided by the mean size of the instar; head capsule widths were 0.38, 0.51, 0.68, and 0.81 mm for 1st, 2nd, 3rd, and 4th instars, respectively (based on Lundgren et al., 2005). These comparisons were contingent on all stadia being offered a food species for a standard amount of time (i.e., consumption rate), a parameter which was controlled for the pollen- and *A. glycines*-fed treatments. Because different quantities of *L. decemlineata* eggs and *S. cerevisiae* were given to larvae of different stadia, these treatments were omitted from this analysis. The mean initial meal sizes and consumption indices were compared among the four stadia using the non-parametric Kruskal–Wallis ANOVA; each food was analyzed separately. The Mann–Whitney U test was then applied to separate significantly different pairs of means.

Table 1 Reaction conditions and amplicon information for the PCR primers sequences used to amplify DNA from the four foods consumed by *Coleomegilla maculata*.

Species	Primer sequence	Targeted gene	Product size (bp)	PCR cycles	Primer concentrations (nM)	Annealing temperatures	Dissociation temperature (°C)	References/Genbank accession # for sequenced gene
<i>Aphis glycines</i>	FWD 5'-TTGTACAATCTTAATATAATACCA-3'	COI	255	75	100	54	73.0	Harwood et al. (2007); EF467229
	REV 5'-AGAATAAGGATCTCCCCAC-3'							
<i>Leptinotarsa decemlineata</i>	FWD 5'-CCTTTTCTTGGGCGATTAT-3'	COI	214	65	225	54	74.3	Greenstone et al. (2007); Weber and Lundgren (2009b); AY613926
	REV 5'-TTATCCCAATCCAGGTAGAAAT-3'							
<i>Saccharomyces cerevisiae</i>	FWD 5'-GCAATCGGGCCAGAGGTTA-3'	ITS-1, 5.8S rRNA, ITS-2	150	50	150	51	78.5 and 80.8 (bimodal)	Unpublished; EU789403
	REV 5'-ATTCACATTACGTATCGCATTTC-3'							
<i>Zea mays</i>	FWD 5'-GGCTTCTGATCTCAITCGTATT-3'	COI	141	65	225	56	77.7	Xiao et al. (2006); #AF542203
	REV 5'-AGGGCCCTCGGCATCTCTATTTC-3'							

Table 2

Comparison of consumption indices of *Coleomegilla maculata* larvae in four stadia fed prey and non-prey foods. Within each row, numbers in parentheses indicate sample size, and statistically different means (pair-wise Mann–Whitney *U* tests, $\alpha = 0.05$) are followed by different capital letters.

Food	Consumption index (mean \pm SEM; $100 \times [\text{Ct}^{-1}/\text{head width}]$)				Statistical comparison
	1st instar	2nd instar	3rd instar	4th instar	
<i>Zea mays</i> pollen	6.10 \pm 0.52 (11)A	5.12 \pm 0.40 (12)A	4.45 \pm 0.27 (13)A	3.72 \pm 0.22 (15)B	$\chi^2_3 = 16.94, P = 0.001$
<i>Aphis glycines</i>	4.93 \pm 0.18 (10)A	3.66 \pm 0.17 (13)B	2.66 \pm 0.08 (15)C	2.28 \pm 0.10 (17)D	$\chi^2_3 = 42.14, P < 0.001$

The effect of time and instar on the amount of detectable DNA marker was determined for all treatments. For each food, the initial amount consumed (at $t = 0$) was standardized for all instars according to the initial amount consumed by 1st instars; i.e., each value within an instar was adjusted by a constant, determined as the difference between means of the instar and 1st instars at $t = 0$. This procedure removed variability attributable to differences in the initial amount of food consumed. An independent, two-factor ANOVA was performed for each food with time and instar as independent variables to analyze their effects on the amount of detectable DNA marker (\log of Ct^{-1}). For those foods with a significant effect of instar, a univariate ANOVA was conducted on the normalized data, followed by means separation with Fisher's LSD comparison. For presentation purposes, linear regressions were fitted to the data for each instar fed each food.

3. Results

3.1. Consumption indices and larval age

Invariably, the consumption index of larvae fed *A. glycines* or *Z. mays* pollen decreased with increasing age (Table 2). In all cases, 1st instars had the highest consumption index, and 4th instars had significantly lower consumption indices than all younger instars.

Initial meal sizes followed a trend different from that of the consumption indices. 4th instars fed pollen ate a larger initial meal than younger instars (*Z. mays* pollen: $\chi^2_3 = 12.52, P = 0.01$). Third and 4th instars consumed significantly more *Z. mays* pollen than 1st instars; 2nd instars consumed quantities that were statistically equivalent to all other instars. Larvae of all ages consumed a similar amount of *A. glycines* DNA during the 5-min feeding interval (*A. glycines*: $\chi^2_3 = 0.52, P = 0.92$).

3.2. Comparison of digestion rates

Time had an effect on amount of detectable marker for all four foods, and the digestibility of three of them varied with instar (Fig. 1A–D). In Fig. 1, all of the stadium-specific datasets for each food are adjusted to provide a standard initial quantity consumed; with this presentation, different digestion rates by larvae in each stadium are visualized. For larvae fed *L. decemlineata* eggs, there was a significant effect of larval age (instar) and digestion time on the amount of prey marker detectable in their stomachs (instar: $F_{3,253} = 7.13, P < 0.001$; time: $F_{6,253} = 5.45, P < 0.001$; interaction: $F_{18,253} = 0.41, P = 0.99$). Third instars digested more prey than any of the other instars, and first, second, and fourth instars digested *L. decemlineata* DNA at similar rates. For larvae fed *A. glycines*, there was no significant effect of larval age, but there was a significant effect of digestion time on the amount of prey marker detectable (instar: $F_{3,276} = 2.22, P = 0.09$; time: $F_{6,276} = 11.85, P < 0.0001$; interaction: $F_{18,276} = 0.63, P = 0.87$). For larvae fed *Z. mays* pollen, there were marginally significant effects of instar and time on the amount of food marker found in their stomachs (instar: $F_{3,271} = 2.47, P = 0.06$; time: $F_{6,271} = 2.03, P = 0.06$; interaction: $F_{18,271} = 0.78, P = 0.73$). Fourth instars digested significantly more pollen than did 1st instars, and all other instars digested similar quantities. For larvae fed *S. cerevisiae*, there were significant

effects of instar and time on the amount of detectable DNA (instar: $F_{3,250} = 7.07, P < 0.001$; time: $F_{6,250} = 2.14, P = 0.05$; interaction: $F_{18,250} = 0.85, P = 0.64$). Fourth instars digested significantly more *S. cerevisiae* DNA marker than all of the other instars, which digested similar quantities of the marker. It was evident from the large numbers of dissociation peaks that the *S. cerevisiae* primers amplified DNA from a diverse microbial community present in the guts. Yeast DNA in unfed larvae produced a Ct of 33.30 cycles.

4. Discussion

These results indicate that the digestive capabilities of *C. maculata* larvae increase as they age to allow them to digest food more efficiently. Behaviorally, *C. maculata* larvae consume less food per unit time in proportion to their body size as they age. This suggests that this species does not display compensatory consumption to accommodate the increased food quantities and nutrients needed by the older instars. Rather, the older instars digested the food more efficiently, including the less suitable foods (e.g., pollen and yeast). Thus, qPCR-based gut analysis of a food's DNA is an effective method for monitoring changes in digestion and consumption in animals, and can contribute to our understanding of how a species changes its dietary breadth over its life.

4.1. Consumption as a function of instar

C. maculata consumed both prey and non-prey foods more slowly relative to their body size as they aged (Table 2), which is in contrast to many other studies on insects. Using gravimetric methods, Lundgren and Wiedenmann (2004) found a similar pattern in the consumption index of *C. maculata* larvae fed *Z. mays* pollen, but other foods (e.g. prey) were not examined. Usually the food consumption index increases with larval age since most growth and food consumption occurs during the last stadia (Waldbauer, 1968; Scriber and Slansky, 1981). Certainly, larvae of *C. maculata* consumed more food as they aged, but not proportional to their body size. A partial exception to this was larvae fed *A. glycines*, which consumed similar amount of aphid DNA throughout their larval life. This was unexpected, since Coccinellini are commonly regarded as specialist aphidophages and thus should be well-adapted to aphidophagy (Evans, 2008), though *C. maculata* belongs to a clade of known omnivores within this grouping (Giorgi et al., 2009). *A. glycines* may not have contained sufficient levels of the proper stimuli to elicit increased consumption by *C. maculata*, or it is possible that the documented anti-predator defenses of *A. glycines* (Butler and O'Neil, 2006) may have restricted consumption; but note that pollen is also sometimes defended from predation (Lundgren, 2009a).

4.2. Rate of digestion as a function of instar

C. maculata larvae of successive instars varied in the speed at which they digested three of four foods (all but *A. glycines*), and our data is consistent with the hypothesis that the dietary breadth of *C. maculata* larvae increases with age. Third instars were superior to all others in their ability to digest *L. decemlineata* eggs (Fig. 1A), and

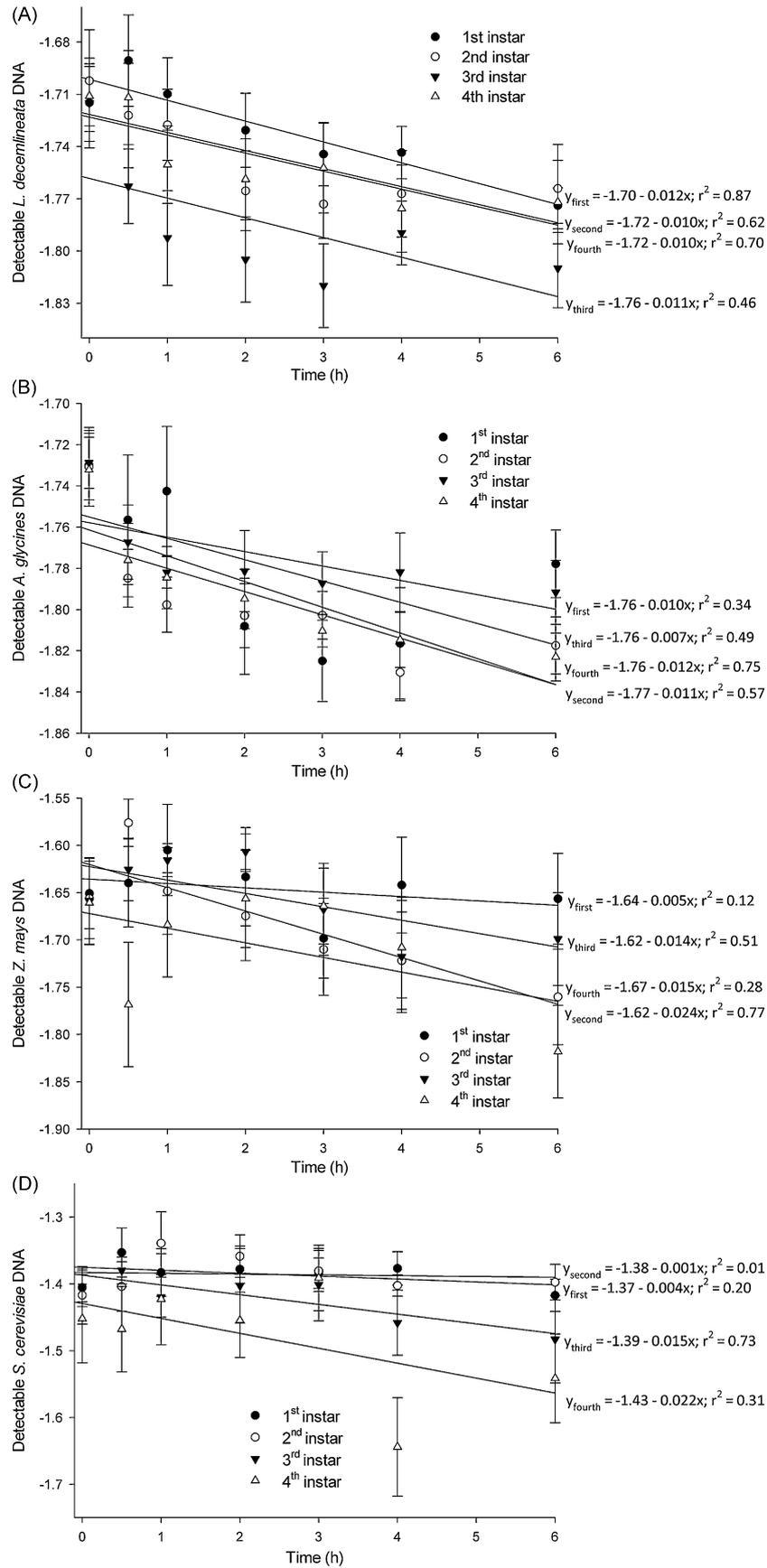


Fig. 1. Relationships between the mean (\pm SEM) quantity of detectable food and time for four larval stadia of *Coleomegilla maculata*. The quantity of detectable food is presented as $\log(\text{Ct}^{-1})$, where Ct is the cycle threshold, and mean values at $t = 0$ for the instars are adjusted (or normalized) according to the mean value for 1st instars at $t = 0$.

4th instars digested the non-prey foods (pollen and yeast) faster than some earlier instars (Fig. 1C and D). The increased ability of later instars to digest *Z. mays* pollen DNA is consistent with a previous study that examined whole-pollen digestion and assimilation (Lundgren and Wiedenmann, 2004). The results have important evolutionary implications for the life history strategy of *C. maculata*, and may suggest that this zoophytophagous insect broadens its diet in order to accommodate increased nutritional needs of older instars. Aphids are equally digestible to all instars, although they were not consumed at a greater rate by older instars. Aphids are known to be of limited nutritional value to some predators (Toft, 2005), and *C. maculata* possibly adjusts its diet to optimize nutrition. Physiological mechanisms that improve digestion of non-prey foods during the final stadium are still unknown.

Dietary breadth of some zoophytophagous insects changes over their larval lives, but the pattern seen in the current study differs from that seen in another omnivorous group. In some zoophytophagous Heteroptera, the youngest instars utilize plant-based foods better than older ones. Young bugs are poor at dispersing, and their nutrition is largely dependent on the oviposition sites selected by their mothers. For *Orius insidiosus*, reproductive decisions are driven by host plant characteristics and quality (Lundgren et al., 2008; Lundgren et al., 2009b), and young nymphs rely on plant-based nutrition until they can locate prey. In contrast, young *C. maculata* in the current study were less efficient at using plant-based resources. Undoubtedly, the increased intake, digestion, and efficiency of conversion of pollen and yeast by *C. maculata* during later stadia are related to the requirements of alternative resources by older larvae. Differences in the ontogenetic changes in dietary breadth between *C. maculata* and some predatory Heteroptera may be partially reflective of their relative capabilities at reducing local populations of prey.

This study discovered a high level of background *Saccharomyces* DNA was present in the guts of *C. maculata*, and suggests that a rich yeast community of unknown function resides within the guts of *C. maculata* larvae. *C. maculata* and other coccinellids are known to consume fungal material in the field (Forbes, 1883; Triltsch, 1999; Sutherland and Parrella, 2009) and can survive on *S. cerevisiae*-based diets in the laboratory (Nijima et al., 1997; Weber, personal observation). Nutritional symbiosis with microbes (and yeasts in particular) are documented in predatory beetles and other entomophagous insects (Woolfolk et al., 2004; Gibson and Hunter, 2005; Vega and Dowd, 2005; Lundgren et al., 2007; Lehman et al., 2009; Lundgren, 2009a), but have not been well explored for Coccinellidae. A more comprehensive examination may reveal important mutualistic interactions between the microbial community initially noted here and *C. maculata*. It may even be that the physiological conditions of the gut that foster the enteric microbial community contributed to the slow digestion of ingested *S. cerevisiae*.

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