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SHORT COMMUNICATION

Tracking predation of subterranean pests: digestion of corn rootworm DNA by a generalist mite

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qPCR is a useful tool for understanding predator–prey relationships. We investigated rootworm DNA digestion by male and female predatory mites. Males and females initially consumed comparable amounts of DNA, which was digested at similar rates. Field-collected mites need to be preserved quickly for best qPCR results.

Keywords: quantitative PCR; gut content analysis; western corn rootworm; *Diabrotica*; soil mite

One challenge in studying predator–prey relationships in subterranean environments is the inability to directly observe predation events. Laboratory feeding trials have merit, but their conclusions are strengthened by the addition of field-based information from genetic gut content analysis. Quantitative or real-time PCR (qPCR) can be used to confirm which predators have eaten a target prey species and determine how much prey DNA is present within their gut (Zhang, Lü, Wan, and Lövei 2007; Lundgren, Ellsbury, and Prischmann 2009a). However, a critical first step in interpreting field-based gut content analysis is estimating the relative rates at which predators digest prey DNA (Greenstone, Rowley, Weber, Payton, and Hawthorne 2007; King, Read, Traugott, and Symondson 2008).

Corn rootworms are widespread economic pests of maize (*Zea mays* L.), with all three larval instars feeding underground on corn roots (Spencer, Hibbard, Moeser, and Onstad 2009). Immature stages are attacked by a suite of natural enemies (Lundgren et al. 2009a, Lundgren, Nichols, Prischmann, and Ellsbury 2009b; Toepfer et al. 2009), including *Gaeolaelaps* (*Hypoaspis*) *aculeifer* (Canestrini) (Mesostigmatidae: Laelapidae), a cosmopolitan soil-dwelling mite (Prischmann, Knutson, Dashiell, and Lundgren 2011). The objectives of this study were to determine the rate at which *G. aculeifer* adults digest rootworm DNA and to compare digestion rates between male and female mites.

Gaeolaelaps aculeifer were purchased from Koppert Biological Systems (Romulus, MI). Mites and their associated shipping material (i.e., vermiculite and astigmatid feeder mites) were transferred to plastic containers and maintained in an incubator (16 h L:8 h D, $25 \pm 2^{\circ}$C, 40–60% RH). Mites were starved for 48–72 h prior to experiments. Western corn rootworms (Coleoptera: Chrysomelidae:

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Diabrotica virgifera virgifera LeConte, non-diapausing strain) were supplied as eggs (USDA-ARS NCARL, Brookings, SD), although neonate larvae were used in feeding trials.

Rootworm DNA digestion curves were determined for *G. aculeifer* based on methods developed for carabids, phalangiids, and spiders (Lundgren et al. 2009a). Experimental arenas consisted of autoclaved 2.0-mL microtubes filled with 1.0 mL of moist plaster. Individual mites were transferred to arenas using alcohol and heat-sterilized forceps. Each arena contained 5–10 newly hatched, unfed first instar rootworms. Only mites that fed continuously on the same prey item for 5 min were used in DNA digestion assays. Exploratory attacks (e.g. when predators briefly penetrated prey, but did not keep feeding) were omitted from analyses. After feeding, mites were transferred to sterile 2.0-mL microcentrifuge tubes with moist cotton in the lid, randomly assigned to a treatment group, and held at $25 \pm 2^{\circ}C$ in constant darkness. Predators were killed at 0, 0.5, 1, 2, 4, 6, 8, 24, or 48 h after feeding by filling tubes with pre-chilled 70% EtOH and freezing at –20°C (Weber and Lundgren 2009). Negative controls consisting of unfed mites were processed in the same manner to monitor potential background levels of rootworm DNA (i.e. contamination). Treatments were replicated 10 times.

All mites (those fed rootworms and unfed negative controls) and positive rootworm controls ($n = 15$ unfed rootworm first instars) were macerated in microcentrifuge tubes using sterile pestles and DNA extracted using DNeasy Blood & Tissue Kits (#69506, Qiagen Inc., Valencia, CA). DNA was stored at $-20^{\circ}C$. PCR reactions were conducted using a MX3000P™ qPCR system (Stratagene, La Jolla, CA), primers that amplify a *D. v. virgifera*-specific DNA sequence (Genbank accession #AF278549, Lundgren et al. 2009a), and Brilliant® SYBR Green qPCR Master Mix (Qiagen). SYBR Green binds to double-stranded DNA and fluoresces. The PCR cycle at which fluorescence can be detected above background levels is the threshold cycle, or Ct value. The lower the Ct value, the earlier DNA was detected, and the higher the amount of target DNA present in the sample. For additional details on DNA analyses see Lundgren et al. (2009a).

Mean Ct values were standardized to normalize plate to plate variability based on how intraplate rootworm positive control values deviated from the overall (inter-plate) positive control mean. Linear regressions of rootworm DNA (Ct^{-1} values) over time were created for male and female *G. aculeifer*. Initial meal size consumed (mean Ct^{-1} at 0 h post-feeding) was compared between female and male mites using *t*-tests. To compare digestion rates among male and female *G. aculeifer*, initial meal sizes were normalized and Ct^{-1} values compared using ANCOVA, with sex as the main effect and time as a covariate. Background levels of DNA in unfed controls were compared to DNA levels found in fed mites using *t*-tests. These analyses allowed us to determine how much rootworm DNA mites initially ingested, how quickly prey DNA was digested, and if male and female *G. aculeifer* digested prey DNA at different rates.

Positive rootworm controls had a mean Ct^{-1} value of 0.070 ± 0.001. The amount of rootworm DNA initially consumed by both female and male *G. aculeifer* in 5 min was approximately half that within the rootworm control ($♀Ct^{-1} = 0.036 \pm 0.003$, $♂Ct^{-1} = 0.032 \pm 0.001$), and although the mean initial meal size was slightly higher in females, this difference was not statistically significant ($df = 1,18$, $P = 0.152$). Initial DNA ingested by *G. aculeifer* was comparable to the amount consumed by

Cyclotrachelus alternans (Casey) (Coleoptera: Carabidae) feeding on rootworm third instars for the same length of time (Lundgren et al. 2009a). Time was significantly and negatively correlated with the amount of detectable rootworm DNA (df = 1,175, $P < 0.001$; Figure 1). Female and male *G. aculeifer* digested rootworm DNA at similar rates (df = 1,175, $P = 0.316$). At 48 h the amount of rootworm DNA recovered from fed females (48 h $♀Ct^{-1} = 0.025 \pm 0.002$) was still significantly higher than unfed controls (unfed $♀Ct^{-1} = 0.012 \pm 0.005$; df = 1,18, $P = 0.020$). At 24 h the amount of DNA recovered from fed males (24 h $♂Ct^{-1} = 0.023 \pm 0.002$) was not significantly different than unfed controls, although the *P*-value was marginal (unfed $♂Ct^{-1} = 0.014 \pm 0.005$; df = 1,18, $P = 0.078$). There was no significant difference between sexes in the amount of background DNA associated with unfed controls, which was due to surface contamination of a few individuals ($♀Ct^{-1} = 0.012 \pm 0.005$, $♂Ct^{-1} = 0.014 \pm 0.005$; df = 1,18, $P = 0.737$). Potential contamination may have contributed to data variability for experimental mites, however, linear relationships between mean prey DNA and time were strong ($♀R^2 = 0.76$, $♂R^2 = 0.88$; Figure 1).

DNA-based techniques are important tools that can be used to elucidate trophic interactions in subterranean environments, including mite consumption of nematodes (Read, Sheppard, Bruford, Glen, and Symondson 2006; Heidemann, Scheu, Ruess, and Maraun 2011) and fungi (Remén, Krüger, and Cassel-Lundhagen 2010). Our results indicate that *G. aculeifer* must be collected and preserved relatively soon after feeding in order to detect predation using qPCR techniques. This parallels results from Read et al. (2006) using a similar-sized laelapid mite (*Stratiolaelaps miles*; Prischmann et al. 2011) eating nematodes. However, temperature can impact DNA digestion rates (von Berg, Traugott, Symondson, and Scheu 2008), thus the ability to detect predation in field-collected soil mites is likely greater than our study indicates. Surface contamination by prey DNA needs to be considered when using PCR techniques (Remén et al. 2010), especially when arthropods regurgitate gut contents (Read et al. 2006) or employ reflexive bleeding as defensive strategies (Lundgren, Toepfer, Haye, and Kuhlmann 2010).

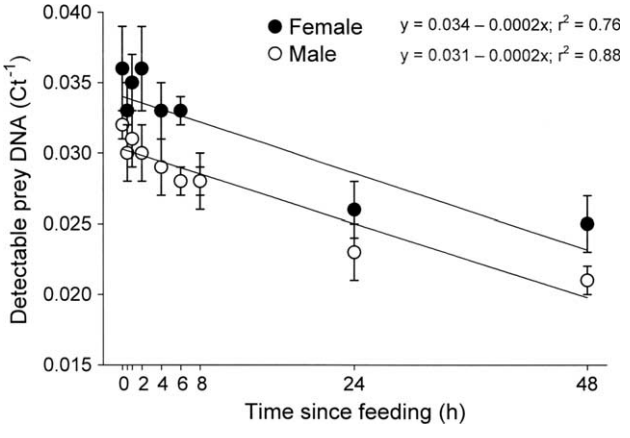

Figure 1. Digestion rates of rootworm DNA by *G. aculeifer* females and males over 48 h. Mites fed on a first instar *D. v. virgifera* for 5 min. Ct^{-1} represents the amount of detectable prey DNA within the predator. Data are means ± standard errors.

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